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Article



# Analysis of Covarine Particle in Toothpaste Through Microfluidic Simulation, Experimental Validation, and Electrical Impedance Spectroscopy

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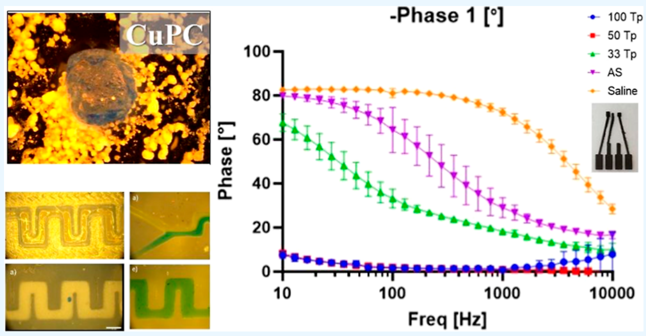
**ABSTRACT:** Covarine, copper phthalocyanine, a novel tooth whitening ingredient, has been incorporated into various toothpaste formulations using diverse technologies such as larger flakes, two-phase pastes, and microbeads. In this study, we investigated the behavior of covarine microbeads (200 $\mu$m) in Colgate advanced white toothpaste when mixed with artificial and real saliva. Our analysis utilized a custom-designed microfluidic mixer with 400 $\mu$m wide channels arranged in serpentine patterns, featuring a Y-shaped design for saliva and toothpaste flow. The mixer, fabricated using stereolithography 3D printing technology, incorporated a flexible transparent resin (Formlabs' Flexible 80A resin) and PMMA layers. COMSOL simulations were performed by utilizing parameters extracted from toothpaste and saliva datasheets, supplemented by laboratory measurements, to enhance simulation accuracy. Experimental assessments encompassing the behavior of covarine particles were conducted using an optical profilometer. Viscosity tests and electrical impedance spectroscopy employing recently developed all-carbon electrodes were employed to analyze different toothpaste dilutions. The integration of experimental data from microfluidic chips with computational simulations offers thorough insights into the interactions of covarine particles with saliva and the formation of microfilms on enamel surfaces.




## 1. INTRODUCTION

Dental care practices play a central role in maintaining oral health. Toothpaste, an essential part of daily oral care routines, serves multiple purposes such as cleaning, protecting, and improving the appearance of teeth. There has been significant progress in toothpaste formulations, incorporating a wide range of ingredients to address different oral health issues, such as plaque removal, cavity prevention, management of gingival inflammation, and tooth whitening.[1] Over the course of thousands of years since their inception, toothpaste formulations have transitioned significantly from rudimentary concoctions involving, for example, crushed eggshells or ashes to intricate compositions boasting over 20 ingredients.[2] These formulations may include compounds targeting dental caries, periodontal disease, malodor, calculus, erosion, and dentin hypersensitivity. Additionally, toothpaste incorporates abrasives to cleanse and brighten teeth, flavors to refresh breath, and dyes for aesthetic appeal. The effectiveness of toothpaste hinges on its ability to maximize the bioavailability of active ingredients. However, achieving this can be challenging, especially when multiple active agents are integrated into a single formulation. The development of toothpaste remains an ongoing endeavor, as numerous challenges persist, notably the limited oral retention of many active components.[3]

Among these components, pigments and dyes are frequently utilized to enhance the visual appeal of toothpaste and improve the perception of its effectiveness in oral hygiene. The inclusion of colorants, dyes, and pigments in toothpaste formulations represents a complex approach in the field of oral care product development.[4] These chromatic additives serve a dual purpose, synergistically enhancing both the visual appeal and functional properties of toothpaste formulations. In terms of aesthetics, they provide vibrant and attractive colors, thereby enhancing the visual appeal and user attraction to the product. Simultaneously, these chromatic entities, when carefully selected, have the potential to offer therapeutic benefits such

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as optical whitening or the modulation of reflective properties, thereby enhancing the cosmetic aspect of oral hygiene. The combination of aesthetics and therapeutic efficacy emphasizes the significant role of colorants in advancing the modern paradigm of toothpaste design and highlights their importance in promoting user engagement and oral health.[5] The incorporation of pigments and dyes into toothpaste formulations is a well-established practice, yet the precise mechanisms governing their deposition on tooth surfaces and subsequent optical effects remain unclear. These pigments are introduced in various forms, including flakes, microbeads, and grains, each with its own distinct physical characteristics and potential interactions within the oral environment. Consequently, a question arises: what exactly happens to these microbeads and how do they behave when toothpaste comes into contact with saliva, the universal fluid in the oral cavity?

Toothpastes designed for teeth whitening typically achieve this benefit by preventing and removing surface stains. It is extensively documented that using toothpaste with extremely low abrasiveness can lead to the accumulation of stained pellicle on tooth surfaces.[6−8] It is widely accepted that toothpaste requires a certain level of abrasiveness to effectively eliminate or prevent these external stains. Furthermore, other ingredients in toothpaste, such as surfactants, polyphosphates, and enzymes, have been investigated in the scientific literature for their role in stain removal and prevention. However, the prevailing evidence suggests that abrasives remain the primary agents for removing stains in toothpaste formulations. According to the mechanism of tooth whitening, tooth whitening toothpastes are categorized into three groups.

(1) Abrasive action: toothpaste formulations contain abrasive ingredients such as hydrated silica,[9] calcium carbonate,[10] dicalcium phosphate dihydrate,[11] calcium pyrophosphate,[12] alumina,[13] perlite,[14] sodium bicarbonate.[15]

(2) Chemical action: whitening effects relies on the chemical effect of the following ingredients: hydrogen peroxide,[16] calcium peroxide,[17] sodium citrate,[18] sodium pyrophosphate,[19] sodium tripolyphosphate,[20] sodium hexametaphosphate,[21] papain.[22]

(3) Optical action: toothpastes with optical effects include pigments like blue covarine, which work by altering the perceived color of teeth.

They achieve this by applying a thin, semitransparent layer of bluish pigment onto the dental surface. This layer promptly changes how light interacts with the teeth, giving the impression of teeth that are visibly lighter and more radiant.[23−25] The concept of covarine-based tooth whitening revolves around the intriguing hypothesis that the deposition of a blue agent onto the tooth's surface can lead to remarkable changes in the optical properties of dental enamel. Specifically, it is postulated that this deposition induces a shift from a yellow to a blue color, resulting in an enhanced perception of tooth whitening. This optical transformation offers an innovative approach to address the aesthetic concerns of individuals seeking a brighter smile.

The aim of this article is to delve deep into the question of covarine particle behavior by employing a comprehensive approach that combines computational simulations using COMSOL Multiphysics software with experimental validation. By unraveling the behavior of covarine particles within a microfluidic (MF) mixer designed for salivary analyte applications, we seek to shed light on the intricate interplay between toothpaste components and saliva.

## 2. MATERIALS AND METHODS

**2.1. Simulation Setup.** In order to conduct the simulation, a standard study, particle tracing for fluid flow, was executed utilizing the software known as COMSOL Multiphysics. This software is widely recognized for its capability to accurately simulate fluid flow and particle behavior. The simulation required the inclusion of several crucial parameters, namely, the size of the particles, the density of the particles' material, and the viscosity of the fluid. All parameter values were measured for the sake of ensuring precise and reliable results, with the exception of the density of the granule material, which was not measured.

**2.2. Microfluidic Mixer Fabrication.** The fabrication process of the MF mixer involved the utilization of stereolithography (SLA), a 3D printing technology renowned for its exceptional level of precision and versatility in the manufacturing of MF devices. SLA enabled the creation of intricate microchannels, chambers, and features within the MF mixer, consequently ensuring the device's ability to perform with utmost reliability. The utilization of SLA printing has been employed to implement a MF mixer, which is based on serpentine patterns. The active layer of the mixer, which is located in the middle, was printed using SLA technology, while a PMMA layer was utilized for the carrier and top coverage. This active layer of the MF mixer comprises two inlets that were specifically designed for the combination of two liquids. At the conclusion of the elongated serpentine mixer, an outlet has been installed. The selection of optically clear materials was made in order to facilitate visual monitoring of fluid flow within the device. The decision to employ SLA as the manufacturing technique was motivated by its compatibility with a wide range of materials, including resins that are biocompatible. This compatibility played a crucial role in the production of MF devices that are biologically compatible, thus proving beneficial for various applications.[26]

For the construction of the MF chip, flexible 80A resin was chosen as the material due to its ability to mimic the flexibility of rubber. This particular choice of material ensured the establishment of a robust seal between the MF chip and the PMMA holder, even in situations in which the flow rates exceeded the designated specifications. As a result, the structural integrity of the device was effectively maintained, guaranteeing its functionality and durability.[27]

**2.3. Microfluidic Mixer Design.** The MF mixer was designed in a distinctive Y-shaped configuration, featuring channels with a width of 400 $\mu$m arranged in a serpentine pattern consisting of 30 serpentines. The inclusion of two separate inlets was crucial for the intended purpose of the device. One inlet was designated for the flow of saliva, while the other served as the entry point for toothpaste and its various dilutions in saliva. To ensure optimal performance, a series of laboratory experiments were conducted to measure and determine the appropriate values for parameters, such as particle size, density, and viscosity.

**2.4. Experimental Analyses.** The investigation of covarine particles within the MF mixer was carried out through utilization of an optical profilometer, which enabled the observation and analysis of their behavior. Additionally, various dilutions of toothpaste were subjected to viscosity tests and electrical impedance spectroscopy (EIS). Specially designed all-carbon electrodes were employed in these experiments to ensure accurate and reliable measurements. The combination of these experimental analyses, complemented by computational simulations, provided a comprehensive understanding of the interactions between covarine particles and saliva.

**2.5. Fabrication of Electrodes.** The process of fabricating electrodes involved the utilization of conductive carbon nanoparticles that were printed onto a substrate using a binder solution. This method of electrode fabrication yielded electrodes composed entirely of carbon, which possessed certain distinguishing characteristics. These electrodes were specifically designed to exhibit exceptional sensitivity and stability when employed in EIS experiments.

**2.6. Preparation of Samples.** In order to prepare the samples for experimentation, a carboxymethyl cellulose-based artificial saliva (AS), developed by the Belgrade Pharmacy Institution, was blended with Colgate advanced white toothpaste (Table 1). This blending process was carried out

**Table 1. Toothpaste Composition**

| Colgate advanced white[28] | |
|---|---|
| ingredient | function |
| aqua | dissolves substances or ingredients |
| hydrated silica | helps clean and polish teeth |
| sorbitol | helps to dissolve the ingredients and prevent drying or hardening keeping the product smooth |
| PEG-12 | prevents liquids from separating |
| sodium bicarbonate | helps clean and polish teeth |
| aroma[a] | freshens breath and improves a product's taste |
| sodium lauryl sulfate | foaming agent to aid in the removal of debris |
| xanthan gum | thickening or stabilizing agent providing consistency to the product |
| cellulose gum | thickening or stabilizing agent providing consistency to the paste |
| sodium fluoride | active ingredient to help prevent cavities |
| sodium saccharin | a nonsugar sweetener enhancing product taste perception |
| limonene | flavor component essential oil providing specific flavor characteristics |
| CI 74160 | provides coloring to a product |
| CI 77891 | provides coloring to a product |

[a]Aroma(>100 ppm): anethole, eucalyptol, limonene, *Mentha arvensis* extract, *Mentha piperita* (peppermint) oil, menthol.

in three distinct configurations, namely: a formulation consisting of 100% AS, a 50−50 mixture of AS and toothpaste, and a combination comprising 66.7% AS and 33% toothpaste. To evaluate the viscosity of these different sample configurations, measurements were conducted under controlled room temperature conditions of 25 °C. To ensure the accuracy of these measurements, a RheolabQC rotary viscosimeter, manufactured by Anton Paar in Graz, Austria, was employed along with suitable viscosimeter tools. We utilized two viscosimeter tools: a cylindrical (CC27) tool, recommended for thick solutions, and a double gap (DG42) tool, suitable for less viscous solutions.

Three healthy volunteers were recruited for the study. The participants were volunteer staff from the Dentistry Department at the University of Novi Sad. The study was approved by the Ethical Committee of the Dental Clinic at Vojvodijna Clinic, Faculty of Medicine, University of Novi Sad. The volunteers gave their informed consent. Saliva was collected using the spitting method and tested within 2 h. The spitting method involved collecting saliva and spitting into a graded test tube every for 60 s. About 5 mL of unstimulated saliva was collected and stored at 4 °C for analysis.

**2.7. Experimental Setup and Data Collection.** The rationale behind capturing three-dimensional profile images of the MF mixer was rooted in an inquiry into the appropriateness of three-dimensional printing for the precise fabrication of MF devices. The SLA three-dimensional printing as an exceptional technology for producing MF mixers was used. Its accuracy, versatility in material selection, and ability to faithfully replicate intricate geometries were pivotal in the investigation. To observe the behavior of covarine particles upon contact with AS, a configuration was utilized, consisting of a three-dimensional optical profilometer and a syringe pump equipped with two syringes—one containing AS and the other filled with toothpaste. In order to obtain highly detailed images, the investigation made use of a Huvitz Bioimager microscope, which possesses magnification capabilities of 25× and 50×. These images were subsequently subjected to rigorous analysis and processing through the utilization of Panasis software, a tool that is widely recognized for its ability to manipulate and measure acquired images. Within the Panasis software platform, we employed 3D profiling techniques for a 3D optimal profile and all-in-focus 2D imaging.

**2.8. EIS Analysis.** Impedance spectroscopy measurements of toothpaste in combination with AS at various ratios were carried out using PalmSense 4 connected to PSTrace 5 software. PalmSense 4, with dimensions measuring 15.7 × 9.7 × 3.5 cm, operated in a three-electrode arrangement. The output voltage ranged from 1 mV to 0.25 V rms (0.7 V peak to peak), and the frequency spanned from 10 $\mu$Hz to 1 MHz. Impedance spectra were recorded within the frequency range of 10 Hz to 1 kHz, utilizing 28 logarithmically spaced points. Five distinct measurements were taken for all samples in order to obtain the impedance spectra. Mean values and standard deviations were analyzed with respect to frequency across all 28 measuring points.

**2.9. Statistical Analysis.** Descriptive statistics were used to represent the data with mean values and standard deviations. The data's normality was assessed using the Shapiro−Wilk test. The significance of differences among the groups was evaluated using the Wilcoxon rank-sum test. For cases with multiple treatments, repeated-measure ANOVA was used with the Durbin Conover pairwise comparison test. The statistical analysis was performed using SPSS 20.0 and Jamovi software (version 2.3.24).[29,30]

## 3. RESULTS

**3.1. Design.** The MF mixer based on serpentine patterns is implemented using SLA printing in layer 2, which contains three inlets for two substances that need to be mixed. At the end of the long serpentine mixer, an outlet for the mixed liquid is installed. Layer 2 (the 3D printed layer) is sandwiched between layers 1 and 3, which are fabricated through micromachining of 6 mm thick PMMA layers; see Figure 1. An illustration of the layers of the constructed mixer can be seen in the image. On the top cover (layer 1), openings are left for PTFE tubes serving as inlets and outlets. These openings


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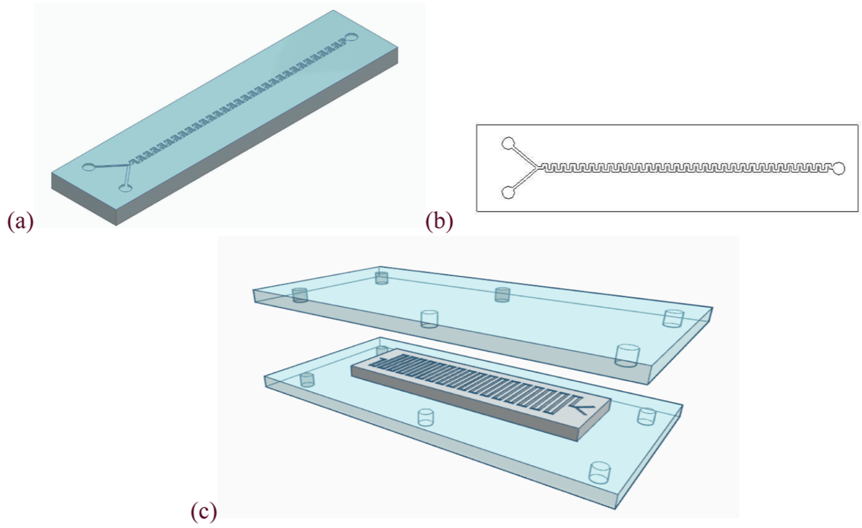


**Figure 1.** Design schematic of MF mixer (a) channel 3D, (b) channel SLA 2D (c) 3D view of PMMA enclosure over SLA channel part.

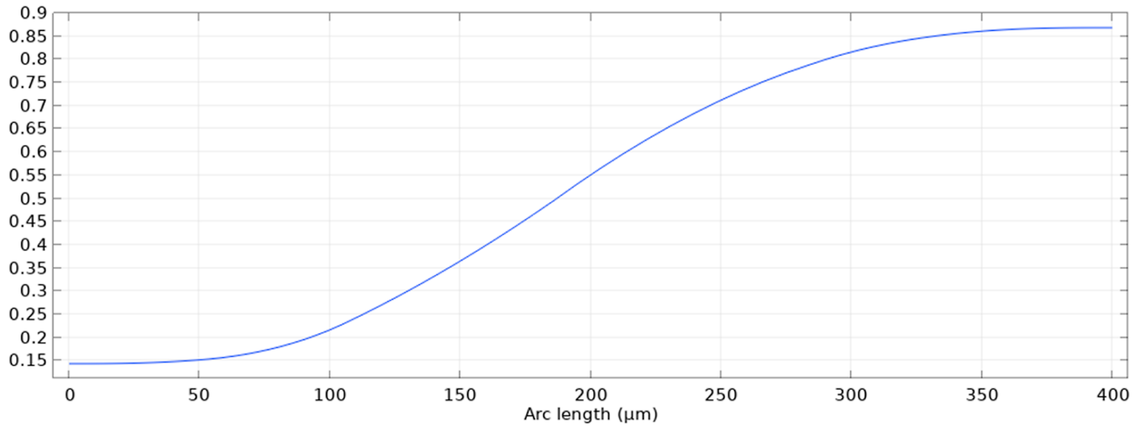


**Figure 2.** Concentration change through cross section of mixer channel on probe 6 for the fluid flow 15 $\mu$L/min.

are made with a diameter that is 10 $\mu$m larger than the PTFE tubes. When installing the PTFE tubes, the outer tube wall is coated with super glue, which perfectly fills the gap between the PMMA and the tube. This ensures a leak-free connection.

**3.2. Fabrication of a Mixer.** The layer height during printing was set to 100 $\mu$m, while other printing parameters were selected for the flexible 80A resin according to the manufacturer's recommendations.[31] The model was printed directly on a mounting plate with upward-facing open channels to eliminate the need for support structures. Afterward, the printing channels were thoroughly rinsed with isopropyl alcohol (IPA, Sigma-Aldrich) and left in IPA for 20 min to ensure the complete removal of any unwanted resin residues. Subsequently, the MF channels were flushed with a syringe filled with pressurized IPA to remove any remaining resin residues inside of the channels. After rinsing, the printed MF chip was left in IPA, placed in an ultrasonic bath at a temperature of 39 °C for 20 min. At this temperature, the uncured resin has the lowest density and can be easily removed from the narrow channels of the MF chip.

**3.3. Simulations.** Mixing efficiency of the mixer was evaluated through several steps. First, COMSOL simulations were computed for the fixed mixer design. Simulations were computed to determine optimal fluid flow for the best mixing of two fluids. Examined fluid flows were 5, 10, 15, 20, and 25 $\mu$L/min. From simulation results, the fluid concentration change was obtained from the cross section at all observing points (probes). Example of concentration through channel cross section can be seen at Figure 2 where on the $x$-axis is displayed geometrical position in the cross section and on the $y$-axis is concentration of fluids. Pure concentration of fluid 1 (AS) is represented as 0, pure concentration of fluid 2 (mixture 33/66) is represented with value 1 and ideal mixture of those 2 is represented with value 0.5.

Values from this graph were taken and the mixing index was calculated as it is described in Podunavac et al.[32]

$$\mathrm{MI} = 1 - \frac{\int_0^{\mathrm{length}} |c - c_\infty|\, \mathrm{d}x}{\int_0^{\mathrm{length}} |c_0 - c_\infty|\, \mathrm{d}x}$$

where length represents diameter of channel, $c$ represents value from the graph, $c$ represents normalized concentration value of ideal mixed fluids (0.5 in this case), and $c_0$ represents the normalized concentration value of pure fluid (in this case it is 1). On the figure below a, mixing results through the whole channel and position of observing points (probes). With results of MI from all probes and for all simulated fluid flows, a heatmap was constructed (figure below b) and concluded that optimal fluid flow for our fluids and our mixer design is 15 $\mu$L/


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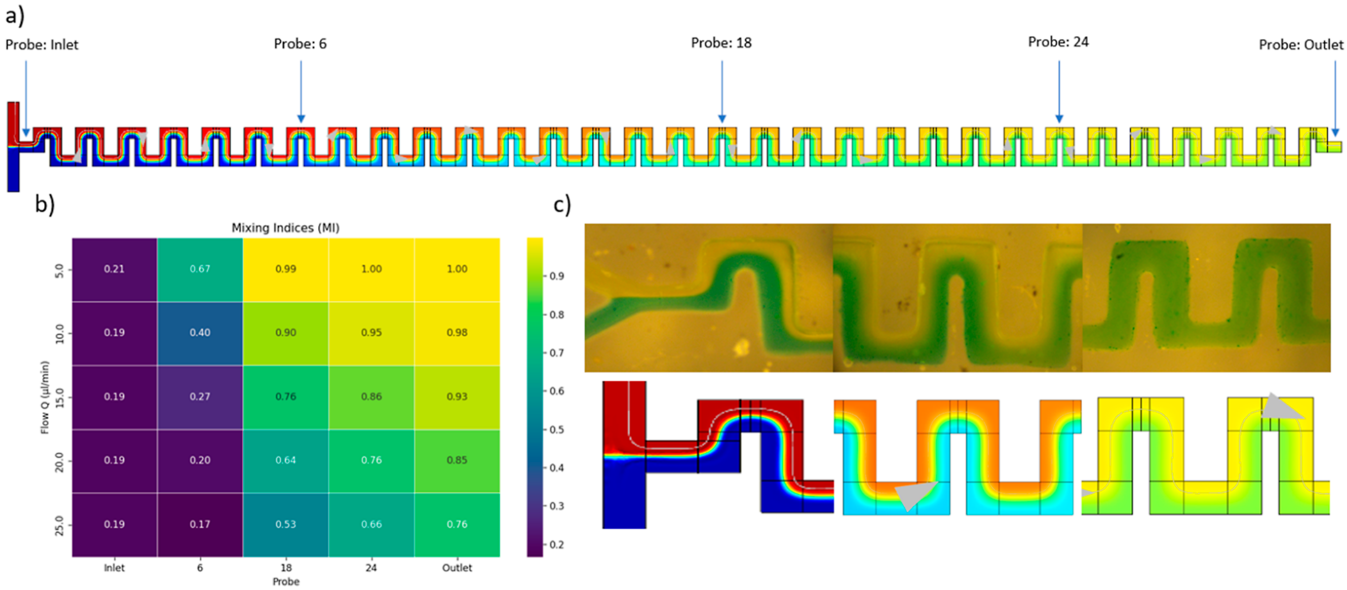


**Figure 3.** (a) Simulation result for flow of 15 $\mu$L/min with marked observing points (probes). (b) Heatmap of mixing indices calculated for every simulated flow, at every observing point. (c) Comparison of simulation results and experimental results of mixing.

**Table 2. Viscosity of a Composition of Toothpaste and AS**

| | 0% AS CC27 | 50% AS CC27 | 66,7% AS CC27 | 100% AS CC27 | 66,7% AS DG42 | 100% AS DG42 |
|---|---|---|---|---|---|---|
| number of values | 30 | 30 | 30 | 30 | 30 | 31 |
| minimum | 3168 | 204.9 | 84.8 | 2.2 | 77.7 | 2.5 |
| maximum | 3982 | 255.8 | 103.9 | 2.9 | 95.4 | 2.6 |
| range | 813.8 | 50.9 | 19.1 | 0.7 | 17.7 | 0.1 |
| mean | 3541 | 227.6 | 93.33 | 2.52 | 85.79 | 2.548 |
| std. deviation | 243.1 | 15.02 | 5.95 | 0.1648 | 5.342 | 0.0508 |
| std. error of mean | 44.38 | 2.742 | 1.086 | 0.0301 | 0.9753 | 0.009124 |

min. To make sure, experimental validation was conducted and results was compared with simulation results as can be seen on figure (below c).

In Figure 3a, simulation results of covarine particle tracing for an observed MF mixer in the 10th second of flow are presented. Results proved that the whole MF mixer is passable and there would not be clogging problems. Also, it can be seen that all particles got in the central flow of the MF mixer and their speed was 7−9 mm/s.

**3.4. Viscosity Measurements.** Based on the provided data, the viscosity measurements of toothpaste, AS, and their mixtures at different ratios were obtained by using a viscosimeter. Table 2 and Figure 4 show the results for each sample, including the number of values (measurements), minimum and maximum values, range, mean, standard

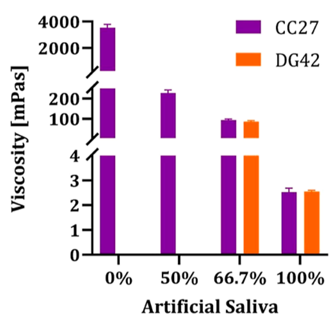


**Figure 4.** Viscosity of a composition of toothpaste and AS.

deviation, and standard error of the mean. For the 0% AS sample, which represents pure toothpaste (CC27), the viscosity ranged from 3168 to 3982, with a mean value of 3541. The standard deviation was 243.1, indicating a moderate level of variability among the measurements. When the toothpaste was mixed with 50% AS (CC27), the viscosity decreased significantly, with values ranging from 204.9 to 255.8. The mean viscosity for this mixture was 227.6, with a lower standard deviation of 15.02, suggesting a higher level of consistency compared to that of the pure toothpaste. For the 66.7% AS (CC27) and 33% toothpaste mixture, the viscosity ranged from 84.8 to 103.9, with a mean value of 93.33. The standard deviation was 5.95, indicating a relatively low variability among the measurements. For the 100% AS sample (CC27), which represents pure AS, the viscosity was considerably lower, ranging from 2.2 to 2.9. The mean viscosity was 2.52, with a small standard deviation of 0.1648, suggesting a high level of consistency in the measurements. It is important to note that for the 100% AS and 66.7% AS samples, the measurements were repeated using a different viscosimeter (DG42). The results for both samples were similar, with viscosities ranging from 77.7 to 95.4 for 66.7% AS (DG42) and from 2.5 to 2.6 for 100% AS (DG42). The mean values were 85.79 and 2.548, respectively, and the standard deviations were 5.342 and 0.0508, indicating relatively low variability among the measurements obtained using a second viscosimeter. In summary, the viscosity measurements of toothpaste, AS, and their mixtures indicate that the addition of AS to toothpaste significantly reduces the viscosity, with the


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highest viscosity observed for pure toothpaste and the lowest viscosity observed for pure AS. The data also suggest that the measurements obtained using the second viscosimeter were consistent with those obtained using the first viscosimeter for the 100% AS and 66.7% AS samples.

**3.5. Experiment—Particle in the Flow.** After simulation results showed that the experiment was possible, physical validation was done. Results have shown almost identical behavior of covarine particles as simulation. Particle size did not change during flow through the whole MF channel, and particles were flowing near the center of the mixer as was expected due to laws of laminar flow. On the Figure 5 below can be seen movement captured as 3 images of the same covarine particle.

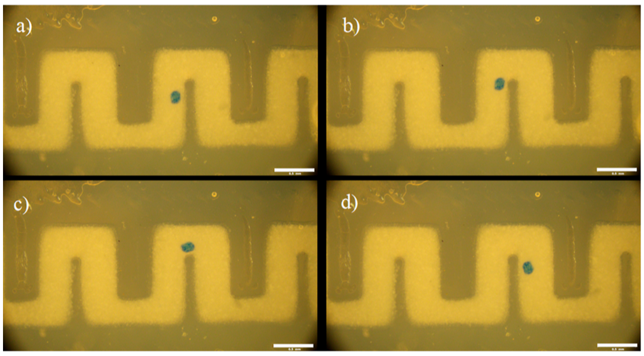


**Figure 5.** Particle trajectory over time, barline 200 $\mu$m.

**3.6. Electrodes.** On the Figure 6, screen-printed all-carbon electrodes are presented. Wider surfaces are designed as pads (contacts) for crocodile clips and are designed to be easy to connect with the PalmSense 4 cable. Narrower surfaces on the other side are designed to be the contact surface for liquid samples. These electrodes are mainly designed to be electrodes for electrochemical measurements, especially EIS. Similar to the standard three-electrode system, our system has working electrode, WE; reference, RE; and the auxiliary electrode, AE. One of the many advantages of this system is that the volume needed for measurement is very low.

**3.7. Electrochemical Impedance Characterization Results.** EIS measurements were done with 3 separate (all carbon) electrodes, with 5 different samples. Used samples were: saline, AS, mixture of 33% toothpaste and 66% AS (33 TP), mixture of 50% toothpaste and 50% AS (50 TP), and pure toothpaste (100 TP). Observed parameters were phase and impedance as functions of frequency, and the results can be seen in Figure 7. Data shown in the figure below are mean values of measured parameters at specific frequency points for each sample from all three electrodes with their standard deviations.

Based on the given data, it can be observed that the impedance ($Z$) of the undiluted toothpaste displays a distinct frequency dependence. At lower frequencies, the impedance is comparatively elevated, implying a hindrance to the passage of the electrical current. Conversely, as the frequency escalates, the impedance decreases, implying an enhanced capacity for electrical conduction. For the combination of toothpaste and AS in equal proportions of 50%, the impedance ($Z$) values exhibit a distinct pattern in comparison to pure toothpaste. Across all frequencies, the impedance of the combination is noticeably lower than that of pure toothpaste, indicating an enhanced level of electrical conductivity. This decrease in impedance implies that the inclusion of AS augments the electrical characteristics of the mixture, which is most likely attributed to the conductive properties of saliva. The significant reduction in impedance mirrors the modified electrical behavior of the combination in contrast with pure toothpaste, accentuating the influence of the saliva component on its electrical properties. The impedance ($Z$) values for the combination of toothpaste and AS with a ratio of 33% toothpaste and 67% AS are situated between the impedance values of pure toothpaste and the 50−50 mixture with AS. This occurrence of intermediate impedance serves as an indicator of a moderate enhancement in electrical conductivity in comparison to that of pure toothpaste. The gradual decline in impedance as the proportion of AS increases implies that the electrical conductivity of the mixture increases with the increase in the proportion of saliva. In contrast to the 50−50 mixture, the 33−67 mixture exhibits a slightly higher impedance across all frequencies, thereby signifying a lower

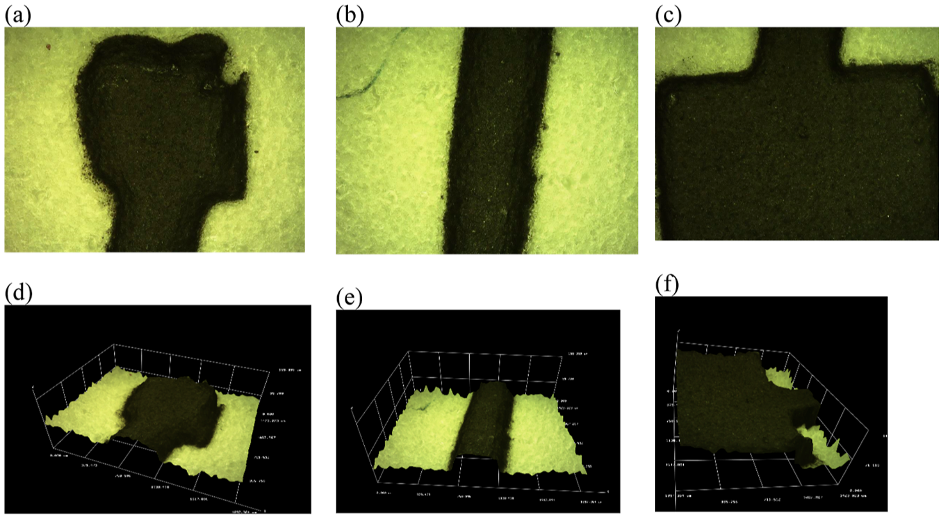


**Figure 6.** Optical microscopic image of the electrode and 3D profile: sensing part (a) 2D, (d) 3D; connection part (b) 2D and (e) 3D, (c) 2D and (f) 3D.



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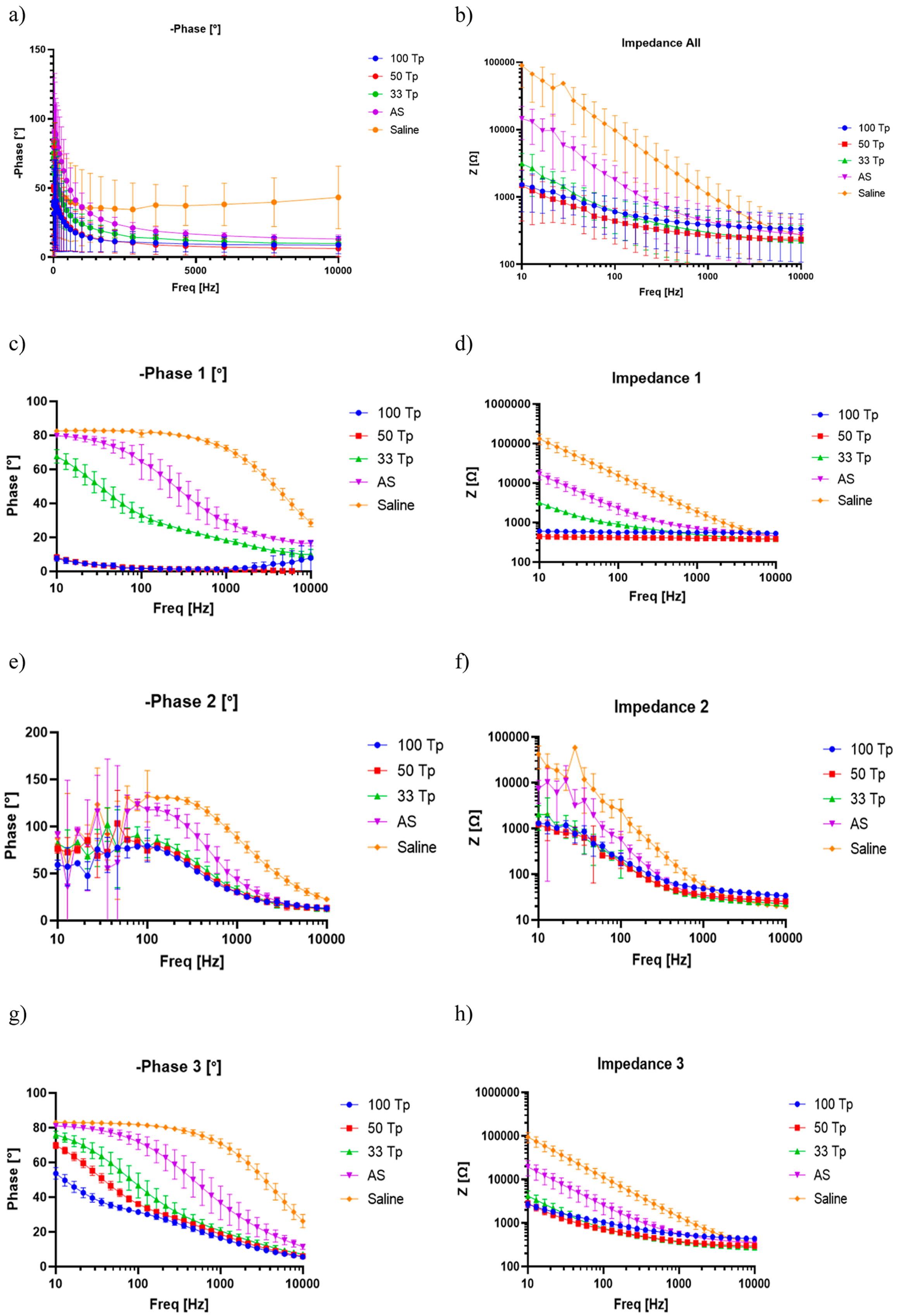


**Figure 7.** EIS average values of phase angle (a) and impedance (b) for all electrodes, average values for phase angle and impedance for electrodes 1, 2, and 3, respectively (c−h).

conductivity than the 50−50 mixture yet still surpassing that of pure toothpaste. This pattern emphasizes the considerable influence of the saliva content on the electrical properties of the mixtures. The impedance ($Z$) values observed in the case



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of pure AS exhibit a consistent trend of an increase in impedance as the frequency rises. This particular behavior is commonly observed in electrolyte solutions, where the impedance increases due to the capacitive effects at the electrode−electrolyte interface as the frequency of the current increases. Drawing a comparison with the previous three analytes, it is evident that pure AS, when compared to a mixture of 50% toothpaste and 50% AS, displays significantly lower impedance values across all frequencies. This observation suggests that the addition of toothpaste to the mixture leads to an increase in the impedance, which in turn indicates a decrease in the electrical conductivity. When pure AS was compared with a mixture consisting of 33% toothpaste and 67% AS, similar trends are observed. Specifically, the 33% toothpaste and 67% AS mixtures demonstrate higher impedance values in comparison to pure AS. The increase in the toothpaste content in the mixture is found to be directly proportional to the increase in the impedance, thereby indicating a reduction in the electrical conductivity. Nevertheless, it should be mentioned that the impedance values in this instance are not higher than those observed in the 50% mixture. This suggests that the higher content of AS in the 33% toothpaste and 67% AS mixture partially mitigates the increase in impedance caused by the addition of toothpaste. A comparison between pure AS and pure toothpaste reveals that pure AS exhibits significantly higher impedance values across all frequencies compared to pure toothpaste. This observation clearly indicates that AS is less conductive than toothpaste. The presence of various electrolytes in saliva contributes to its high electrical conductivity, but when compared with toothpaste samples, toothpaste conductivity was significantly higher. In summary, the comparison conducted in this study sheds light on the influence of the toothpaste content on the electrical properties of the mixtures. It is evident that a higher toothpaste content leads to a decrease in impedance, which directly correlates to an increase in electrical conductivity. On the other hand, the content of toothpaste in the mixtures plays a significant role in enhancing their electrical conductivity, albeit at a level lower than that observed in pure AS. The values of impedance ($Z$) for the saline solution demonstrate a consistent increase in the impedance as the frequency increases, which is a characteristic commonly observed in electrolyte solutions. When the impedance values of the saline solution are compared with those of pure AS, it is evident that the saline solution exhibits significantly higher impedances across all frequencies. This can be attributed to the fact that the saline solution, being a simpler electrolyte, possesses a higher impedance compared with the more intricate composition of AS. This observation suggests that the inclusion of additional components in AS enhances its conductivity in comparison to that of a basic saline solution. When the impedance of the saline solution is compared with mixtures containing toothpaste and AS, it becomes apparent that the impedance of the saline solution is significantly higher than that of the mixtures at all frequencies. This further solidifies the perception that the addition of toothpaste to AS has a noteworthy influence on the electrical characteristics of the mixtures, resulting in a decreased impedance and heightened conductivity. $P$-values for pairwise comparisons were calculated using two-sample $t$ tests. The $p$-value for the comparison between pure toothpaste and a mixture of 50% toothpaste and 50% AS is less than 0.05, indicating statistical significance. Similarly, the $p$-value for the comparison between pure toothpaste and a mixture of 33% toothpaste and 67% AS is less than 0.05, also demonstrating statistical significance. When comparing pure toothpaste to pure AS, the $p$-value is less than 0.05, suggesting statistical significance. Furthermore, the $p$-value for the comparison between pure toothpaste and a saline solution is less than 0.05, indicating statistical significance. Comparing a mixture of 50% toothpaste and 50% AS to a mixture of 33% toothpaste and 67% AS yields a $p$-value of less than 0.05, signifying statistical significance. Similarly, comparing a mixture of 50% toothpaste and 50% AS to pure AS results in a $p$-value of less than 0.05, demonstrating statistical significance. Furthermore, comparing a mixture of 50% toothpaste and 50% AS to a saline solution yields a $p$-value of less than 0.05, suggesting statistical significance. When comparing a mixture of 33% toothpaste and 67% AS to pure AS, the $p$-value is less than 0.05, indicating statistical significance. Similarly, the $p$-value for the comparison between a mixture of 33% toothpaste and 67% AS and a saline solution is less than 0.05, also demonstrating statistical significance. Lastly, when comparing pure AS to a saline solution, the $p$-value is less than 0.05, suggesting statistical significance.

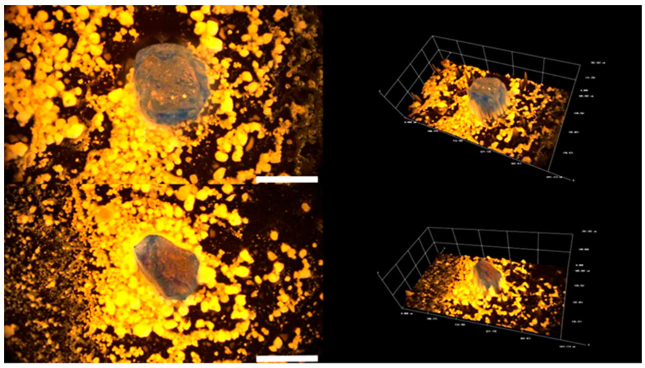

**Figure 8.** Covarine particle size: left, 2D all-in-focus image (barline is 200 $\mu$m), right, 3D optical model.

Based on Figure 11, the phase angle values for pure toothpaste, as given, demonstrate a complex behavior when examined across different frequencies. The phase angle values exhibit a degree of variation throughout the frequency range of 10 to 10,000 Hz. This implies that the electrical properties of the toothpaste are not consistent and undergo changes as the frequency shifts. There are specific frequencies at which the phase angle values experience alterations. The phase angle values do not adhere to a straightforward linear or predictable pattern throughout the frequency range. The variations in the phase angle suggest a combination of capacitive and inductive behavior at different frequencies. Phase angles of 50% tooth paste and 50% AS: the phase angle values for this mixture exemplify a broad spectrum of values, commencing at approximately −4.43° and escalating to around 10.5° as the frequency progresses from 10 to 10,000 Hz. There exists an overarching ascending trend in the phase angles as the frequency augments, indicating a shift from a capacitive to an inductive demeanor. In comparison to pure toothpaste, the phase angles for this combination tend to be inferior, particularly at higher frequencies. This implies that the inclusion of AS influences the electrical characteristics of the mixture, potentially resulting in a more capacitive response.


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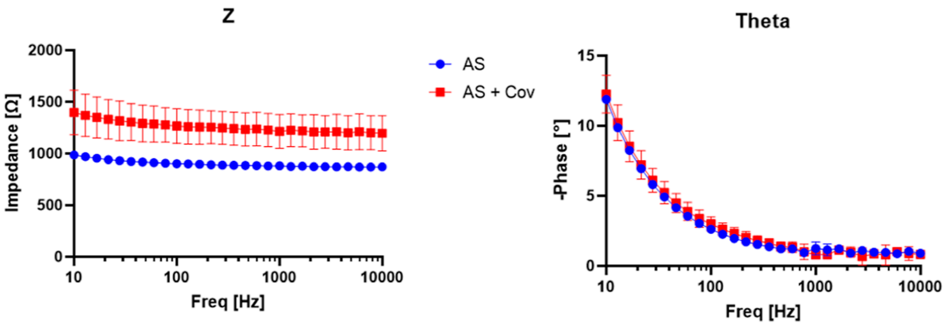


**Figure 9.** EIS average values of impedance (left) and phase angle (right) for pure AS (blue) and of the mixture consisting of AS and covarine particles (red).

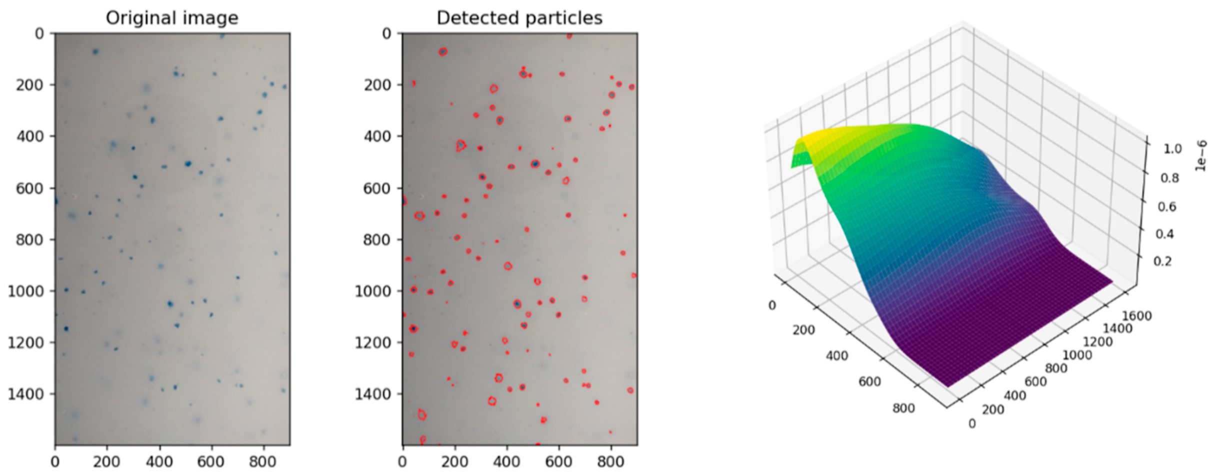


**Figure 10.** Original image of toothpaste 50/50 mixture spread (left), result of HSV detection of covarine particles (middle), and estimated probability density curve (right).

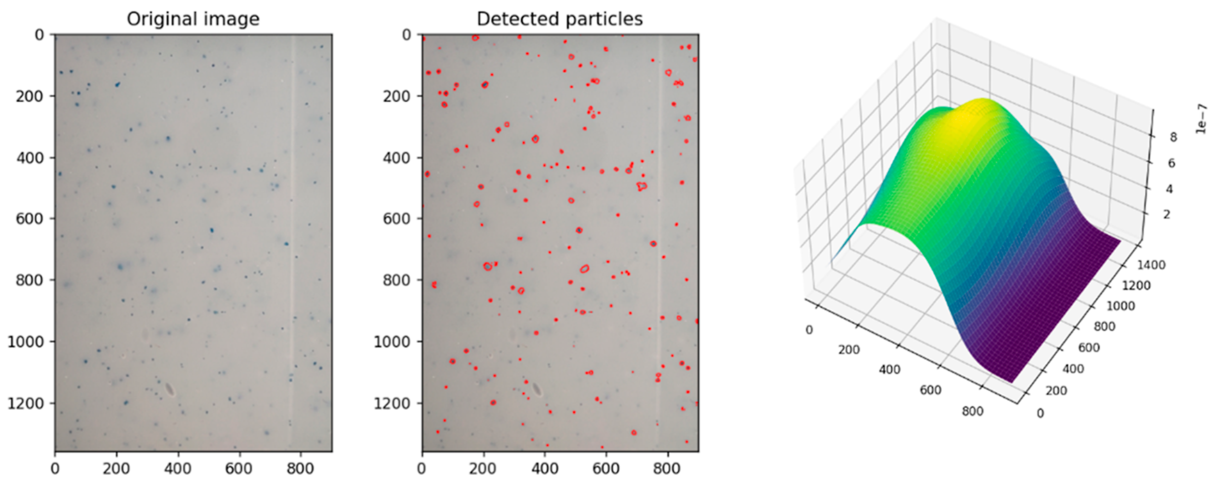


**Figure 11.** Original image of toothpaste 33/66 mixture spread (left), result of HSV detection of covarine particles (middle), and estimated probability density curve (right).

Phase angles of pure toothpaste: the phase angles for pure toothpaste fluctuate between approximately −4.43 and 29.01° throughout the frequency spectrum of 10 to 10,000 Hz. Analogous to the mixture, there exists a general escalating pattern in phase angles with an increase in frequency, signifying a transition from capacitive to inductive behavior. Comparison: the phase angles of the mixture are generally inferior to those of pure toothpaste, suggesting that the addition of AS suppresses the inductive behavior observed in pure toothpaste. Both the mixture and pure toothpaste exhibit similar trends with an increase in frequency, but the mixture demonstrates a more consistent response. The phase angle values for the combination of 33% toothpaste and 67% AS exhibit a range of approximately 7.22 to 73.47° across the frequency spectrum spanning from 10 to 10,000 Hz. An observable trend of increasing phase angles with higher frequencies is evident, implying a transition from capacitive to inductive characteristics. In comparison to both pure


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toothpaste and the mixture containing equal parts of toothpaste and AS, the phase angles for this particular composition are notably elevated, indicating pronounced inductive behavior, particularly at higher frequencies. The phase angle values for pure AS range from approximately 13.56 to 81.04° across the frequency range of 10 to 10,000 Hz. Within the frequency range, the phase angles of pure AS demonstrate a noticeable increase with increasing frequency, indicating a transition from capacitive to inductive behavior. Pure AS exhibits distinct inductive behavior, particularly at higher frequencies. When compared to pure toothpaste, the phase angles of pure AS generally surpass those of pure toothpaste. Although at lower frequencies the phase angles of both pure toothpaste and pure AS are relatively similar, as the frequency increases, AS exhibits a more prominent inductive behavior. Upon comparison with the 50% toothpaste and 50% AS mixture, it becomes evident that the phase angles of pure AS are significantly higher across the entire frequency range. The 50% toothpaste/50% AS mixture shows a much weaker inductive behavior compared to AS, indicating that increasing the proportion of AS substantially enhances the inductive properties of the mixture. In comparison to the 33% toothpaste and 67% AS mixture, pure AS displays higher phase angles, particularly at higher frequencies. This implies that the 33% toothpaste/67% AS mixture exhibits a slightly weaker inductive behavior compared to pure AS despite both having a strong inductive response. To summarize, pure AS exhibits a distinct inductive behavior across the entire frequency range, surpassing both pure toothpaste and the 50% toothpaste/50% AS mixture. Furthermore, the proportion of AS significantly impacts the inductive properties of the mixture with higher proportions resulting in stronger inductive responses.

**3.8. Covarine Particle Size, Uniformity, Distribution, and EIS Analysis.** We performed an examination of particle sizes that encompassed covarine. The 2D as well as 3D visuals demonstrate that particle sizes fluctuate between 100 and 200 μm, both within the paste and in all dilutions (Figure 8). This remains unchanged even subsequent to the micromixer facilitating the flow of the liquid mixture.

In order to quantify the effect of covarine particles within saliva, we performed the following experiment. According to the methodology recently reported by our group,[33] we isolated the covarine particles from the toothpaste as follows:

To commence the preparation of the sample for analysis, we initiated the procedure by introducing 125 mL of toothpaste into a glass beaker possessing a volumetric capacity of 1000 mL (step 1). We generated a concoction by amalgamating this aforementioned volume of 125 mL (step 2) of toothpaste with 800 mL of deionized water, leading to an aggregate volume of 925 mL (step 3). In order to facilitate a comprehensive mixing process, we employed a stirring rod or magnetic stirrer, thereby ensuring uniform dissemination of toothpaste within the aqueous medium. Subsequent to the preparation, we immersed the solution in an ultrasonic bath, utilizing the GT sonic professional ultrasonic cleaner for a duration spanning from 10 to 15 s (step 4). We allowed the solution to undergo the process of sedimentation by leaving it undisturbed for a brief interval of time, approximately 1 min (step 5). Following this, we executed the procedure of decanting (step 6), an act of meticulously extracting the liquid while leaving the sediment behind (step 7). To ensure that the desired substance is isolated, we replicated this process of sedimentation and decanting a total of 5 times. Ultimately, we retrieved the sedimented material utilizing a pipet and intricately transferred it into a glass Petri dish (step 8). Once the sediment congregated, we initiated the drying phase by situating the Petri dish within an oven that was adjusted to a temperature of 60 °C for a period of 3 h or until absolute dryness was attained (step 9). Upon the culmination of the drying stage, we cautiously transferred the desiccated beads into an Eppendorf tube (step 10). As a result, we obtained 130 mg of covarine-containing particles that we used in further analyses.

We performed EIS on the same carbon electrode consistently with the EIS analysis performed with toothpaste mixtures, with three samples of AS and three separate samples of 0.05% mixture of extracted particles and the AS. By examining impedance of covarine particles within AS, we concluded that the impedance has increased, Figure 9. This finding can be explained having in mind that CuPC, despite being a conductor, is likely coated with nonconductive layers, most probably silica. Additionally, these particles may tend to precipitate on the electrode surface.

To determine if mixtures of toothpaste and AS used in our research preserve a uniform distribution of particles, a python algorithm was developed. Samples of mixtures were spread on object glass and images were captured with macroscopic lens. From those images, a representative part with an area of 3 $cm^2$ was cropped, and with HSV detection, covarine particles were detected. HSV detection was useful because of air bubble anomalies that with other detecting algorithms would be detected as particles. After detection, the particle distribution was estimated with kernel density estimation (KDE) with the Gaussian kernel. In the figure below it can be seen that results of KDE estimation have really low value differences at $z$-axis which means that we can consider distribution to be uniform, Figures 10 and 11.

## 4. DISCUSSION

In this study, we focused on several specific aspects to comprehensively evaluate the behavior of covarine particles in Colgate advanced white toothpaste. First, we examined the influence of MF mixing on the dispersion characteristics of the particles. A custom-designed MF mixer was employed, featuring 400 μm wide channels arranged in a serpentine form with 30 serpentines. By precisely controlling the flow of artificial and real saliva and toothpaste through the MF mixer, we aimed to investigate the effect of mixing parameters on the dispersion and uniformity of covarine particles within the toothpaste matrix. In addition to MF mixing, we utilized Comsol simulations to gain insights into the behavior of covarine particles. Parameters such as particle size, density, and viscosity, which were not available in the toothpaste and AS datasheets, were accurately measured in the laboratory to ensure accurate simulations. This computational approach allows for a deeper understanding of the factors influencing the behavior of covarine particles and provides valuable predictions and insights for optimizing toothpaste formulations.

The aspect of MF mixing is significant, as it allows for precise control and manipulation of the flow of toothpaste and saliva, enabling a thorough investigation of covarine particle dispersion characteristics. By studying the influence of MF mixing parameters on the distribution and uniformity of covarine particles within the toothpaste matrix, we can gain insights into the effectiveness of mixing in achieving consistent and optimal dispersion. This aspect contributes to the overall


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understanding of covarine particle behavior by elucidating the role of mixing in determining the whitening efficacy and uniformity of toothpaste formulations. The utilization of Comsol simulations is essential for gaining a deeper understanding of the behavior of covarine particles under different flow conditions. By incorporating parameters specific to covarine particles and toothpaste, we can simulate and study their behavior in a virtual environment. It helps identify critical factors that influence particle dispersion, aggregation, and adhesion, which are difficult to observe directly in experimental settings.

In this study, the SLA 3D printer is used to create the middle layer of the MF chip using a flexible transparent resin. This layer plays a crucial role in ensuring the functionality and performance of the MF mixer. The SLA 3D printer enables the production of intricate designs and contributes to accurate reproduction of the MF chip, ensuring reliable and repeatable experimental results. Transparent PMMA layers are utilized in the study to enclose the MF chip. These layers, cut using a $CO_2$ laser CNC machine, provide a protective housing for the MF chip while allowing visualization of the flow and behavior of covarine particles. The transparent PMMA layers enable researchers to observe the dispersion and interactions of the particles in real time, facilitating the characterization and understanding of the covarine particle behavior. Additionally, the use of transparent PMMA layers ensured the durability and stability of the MF chip during experimental procedures. The optical profilometer is an important tool used to observe and analyze covarine particle behavior during the experiments. It allows for the precise measurement of surface topography and characterization of the covarine particles' distribution. By utilizing an optical profilometer, researchers can quantitatively analyze the formation and properties of microfilms formed by covarine particles. The MF mixer, which is introduced in this investigation and manufactured utilizing SLA 3D printing, represents a notable advancement in the fabrication of MF chips.

Micromixers play a crucial role in lab-on-a-chip devices that find applications in a range of fields such as chemistry and engineering analysis.[34−38] A particular investigation focused on the optimization of a "Y" type electrokinetic turbulent micromixer, revealing that the most effective mixing was achieved in a 350 $\mu$m wide micromixer with specific AC electric field conditions [2]. Another study aimed to develop a rapid and robust method for the structural characterization of monoclonal antibodies. This involved employing microdroplet reactions, automated sample flow injection, and online mass spectrometry analysis [3]. In a separate study, efforts were made to enhance the analysis of isomers and isobars of phospholipids by combining reversed-phase lipid chromatography with an online Paterno−Buchi reaction [4]. Moreover, the utilization of mix-and-inject serial crystallography using X-ray free electron lasers and microcrystalline samples was explored as a technique for capturing atomically detailed snapshots of biomolecules [5]. Finally, MFs were employed for in situ characterization of a chemical extraction process, enabling a comprehensive understanding and analysis of the process conditions at different scales.

Accurate measurement of relevant parameters in the laboratory is of utmost importance in this study to ensure accurate simulations and reliable experimental results. While some parameters, such as toothpaste and AS composition, can be obtained from available datasheets, there are several crucial parameters specific to covarine particles and the toothpaste formulation that need to be measured directly. For instance, the particle size, density, and viscosity are essential parameters that greatly influence the behavior and dispersion of covarine particles. By conducting precise measurements of these parameters in the laboratory, researchers can ensure that the simulations accurately represent real-world conditions and provide reliable predictions of particle behavior under different flow conditions.

This research is expected to make significant contributions to the field of toothpaste formulation and tooth whitening by providing novel insights and advancements. By evaluating the behavior of covarine particles in Colgate advanced white toothpaste, this study aims to uncover crucial insights into their dispersion characteristics, interactions with saliva, and the formation of microfilms on the enamel surface.

The implications for oral health can be seen in the viscosity and mixing properties of both real and AS as well as various mouthwashes and toothpaste. The ability of these substances to coat and lubricate the oral cavity is impacted by their viscosity, which in turn affects the effectiveness of preventive and therapeutic effects. It is crucial to understand the properties and interactions of these substances in order to maintain oral homeostasis and promote oral health.[31,39] The oral cavity is often overlooked as a site for dissolution and mixing due to its rapid transit when food, drinks, and medications are swallowed.[40,41] Viscosity, which represents the resistance of fluid layers to relative movement, is influenced by factors such as intermolecular interactions, molecule size, and shape.[42,43] The deformation of materials under stress or forces, known as rheology, is also relevant in this context. Newtonian viscous fluids, which exhibit linear stress-load rate relationships, accurately represent the behavior of materials like metals, air, water, and oils.[44] The rheological properties of saliva are influenced by multiple factors, resulting in its behavior as a non-Newtonian fluid with decreasing viscosity as shear rate increases.[45] The viscosity of natural saliva varies with shear rate, with significantly higher viscosities observed at rest and shear rates in the range of 0.1−1/s compared to chewing and speech, where shear rates are around 60 and 160 1/s.[43] It is recommended in the literature to test higher shear rates, such as 160 $s^{-1}$ during chewing.[46−49] Furthermore, different shear rates may coexist in the mouth, with some areas experiencing zero shear rate, such as when a bite is held against the cheek.[50] The findings of this study, which did not exceed shear rates of 100/s, align with the majority of previous research, making them suitable for potential data comparisons.[51]

The sizes of particles and pigments in toothpaste can vary depending on the specific formulation and brand. Toothpaste typically contains abrasive particles for cleaning and pigments or dyes for color. The particle size and distribution are important factors in determining the toothpaste's cleaning effectiveness, texture, and appearance. However, the exact specifications may not always be readily available to the general public as companies may consider this information proprietary. The size of the abrasive particles in toothpaste is often in the micrometer range. These particles help in the mechanical cleaning of teeth by removing plaque and stains. Common abrasive particles include silica, calcium carbonate, and aluminum hydroxide. The distribution of these particles can vary, and manufacturers carefully select the size and distribution to balance effective cleaning with minimal enamel


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wear. Pigments and dyes in toothpaste are used for cosmetic purposes, providing visually appealing color. The particle size of the pigments is usually very small, typically in the nanometer range. The distribution of pigments is designed to ensure consistent color throughout the toothpaste. It is important to note that toothpaste formulations can change, and specific information about particle size and distribution may not be disclosed on product packaging. The information shared may be subject to the manufacturer's policies and may not always be publicly disclosed.

The behavior of covarine particles in toothpaste is profoundly influenced by their size and interactions with saliva. The size of the particles plays a crucial role in determining their dispersion and stability within the toothpaste matrix. Smaller particles tend to disperse more uniformly, facilitating better coverage of the tooth surfaces and enhancing the overall whitening effect. On the other hand, larger particles may exhibit uneven distribution, leading to localized whitening and potential clumping. Furthermore, interactions between covarine particles and saliva can affect their adhesion to the tooth surfaces. Saliva, with its complex composition of enzymes, proteins, and electrolytes, can alter the surface properties of the particles, influencing their deposition and subsequent interaction with the enamel surface.

MF devices offer several advantages over conventional laboratory techniques for studying interactions between toothpaste and saliva as well as other biomedical research applications. One of the main advantages of MF devices is their ability to precisely control the flow of fluids and the mixing of different solutions. This allows researchers to study the interaction between toothpaste and saliva in a controlled environment with precise control over the timing, rate, and duration of fluid mixing. This can provide more detailed information about the chemical and physical processes that occur when toothpaste and saliva are combined. Additionally, MF devices can be designed to reduce or eliminate the need for hazardous chemicals or solvents, reducing the environmental impact of laboratory experiments. With the ability to rapidly mix small volumes of fluids, MF devices can enable the screening of large numbers of samples or conditions in a short amount of time. This can be particularly useful for studying the effects of different toothpaste formulations or saliva samples on dental health.

The results depicted in Figure 4 offer insights into the behavior of covarine particles within the observed MF mixer. In the simulated scenario, which took place in the 10th second of flow, it was demonstrated that the entire MF mixer was permeable, thereby indicating the absence of any issues related to clogging. This discovery is of importance, as it suggests that the design of the mixer allows for unobstructed flow, ultimately enhancing its operational efficiency. One of the notable outcome of the simulation was the homogeneous distribution of particles within the central flow of the MF mixer. The particles displayed a consistent velocity ranging between 7 and 9 mm/s. This uniform flow pattern signifies the mixer's ability to maintain a stable laminar flow, a fundamental aspect of MF systems. The observed behavior aligns with the principles governing laminar flow, thereby validating the design of the mixer and its applicability in various MF applications. As already reported, similar MF mixers have various applications in pharmaceutical, biological, and chemical systems. They are used for controlled mixing of multiple samples in MF devices, where the flow is in the laminar regime and mixing is achieved through diffusion and/or chaotic laminar mixing.[52] These mixers are designed to reduce sample consumption and decrease mixing time to microseconds, making them suitable for kinetic studies of biological macromolecules.[53] They are also used in applications such as MF gradient generators, where diffusion is used as the sole mechanism for controlled mixing.[54] Additionally, MF mixers are used in the pharmaceutical, food, and beverage industries for the study of suppression/enhancement of sensorial/chemical effects.[55] Novel methods of mixing enhancement, both passive and active, have been explored to address the challenge of laminar flows in microchannels.[55] These mixers can be integrated into MF devices to enhance the mixing in a controllable manner.

To further support the evidence obtained through simulation, a physical experiment was conducted. The experimental findings closely mirrored the simulated behavior of covarine particles. The particles maintained their size throughout the entire flow within the MF channel, thereby indicating a minimal influence of the channel dimensions on particle size. This consistency holds significance, particularly in applications for which precise particle sizes are important. Additionally, the experimental results confirmed the positioning of the particles near the center of the mixer. This outcome is consistent with the expected behavior dictated by the laws of laminar flow, thereby reinforcing the understanding of the fluid dynamics of the mixer. The close agreement between the simulation and experimental results enhances the confidence in the accuracy of the simulation model. It also underscores the reliability of the mixer and its potential for consistent performance in practical applications. The benefits of using COMSOL simulation in conjunction with experimental validation for the design of MF mixers are as follows. First, highly integrated simulation tools, such as COMSOL, can guide researchers without specialized computational fluid backgrounds to design numerical prototypes of highly integrated devices.[56] Second, numerical simulations can provide insights into the efficiency and time-resolution achievable using MF mixers, allowing for design optimization.[57] Third, simulations can help in understanding the effect of channel occlusion on micromixing leading to improved micromixing efficiency.[58] Lastly, simulations can be used to analyze and predict the mixing effectiveness of different micromixer designs, enabling the development of more efficient and cost-effective devices.[59]

The movement of covarine particles captured in the images (Figure 5) serves as visual evidence of their behavior within the MF mixer. These images constitute a qualitative validation, providing a direct view of the trajectory and positioning of the particles. Such visual confirmation complements the quantitative data obtained from simulations and experiments, thereby enriching the overall understanding of particle behavior in the MF mixer. In conclusion, the collective evidence from simulations, experiments, and visual observations highlights the robustness and efficacy of the examined MF mixer. The absence of clogging issues, uniform particle distribution, and adherence to laminar flow principles underscore the potential of the mixer in various MF applications. These findings not only contribute to the understanding of fluid dynamics within microscale devices but also pave the way for the development of innovative technologies in fields such as biotechnology, medicine, and materials science.

The determination of viscosity in toothpaste, AS, and their various combinations imparts valuable insights into the


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rheological properties of these substances. The data divulge noteworthy alterations in viscosity, as the ratios of toothpaste and AS vary. These findings hold significant implications for practical applications, particularly in the advancement of oral care products. Toothpaste viscosity pertains to the consistency or resistance to movement of toothpaste. The evaluation of toothpaste viscosity holds significance, as it furnishes insight into the flow characteristics and uniformity of the toothpaste. The consistency of toothpaste may be influenced by various factors, such as the concentration and composition of the ingredients used. Research suggests that toothpastes may demonstrate a phenomenon referred to as shear-thinning behavior, which indicates that their viscosity decreases as the rate of shear increases. Techniques such as rheometry and viscometry might be employed for evaluating the viscosity of toothpaste. Grasping the viscosity of toothpaste is vital for devising toothpastes with favorable flow attributes, as well as for predicting their performance during the stages of production, packaging, and utilization.[60]

The viscosity of pure toothpaste (CC27) was discovered to be relatively elevated, with an average value of 3541, signifying dense and gelatinous consistency. This heightened viscosity is inherent to toothpaste formulations and is indispensable for ensuring proper attachment to teeth during brushing. Nevertheless, when toothpaste was blended with 50% AS (CC27), a substantial decline in viscosity was observed, with an average value of 227.6. This reduction implies that the inclusion of AS significantly influences the flow properties of the mixture, potentially leading to easier spreading and an enhanced user experience. Further dilution of toothpaste with 66.7% AS (CC27) yielded a viscosity of 93.33, indicating a further reduction in thickness. This diminished viscosity is desirable for specific oral care applications, such as mouthwashes and gels, where effortless dispensing and rinsing are significant. The pure AS (CC27) exhibited the lowest viscosity with an average value of 2.52, confirming its aqueous and thin consistency. This diminished viscosity is advantageous for applications where a thin and uniform coating is required such as saliva substitutes or moistening agents. The comparison of viscosity measurements between different samples, especially the 100% AS samples measured by using two different viscosimeters (CC27 and DG42), is noteworthy. The comparable viscosity values obtained for the 100% AS samples using both instruments (2.52 and 2.548, respectively) indicate the consistency and reliability of the measurements. This consistency across different instruments fortifies the accuracy of the findings and suggests that the observed viscosity values are robust and reproducible.

The establishment of precise benchmarks for toothpaste viscosity has the potential to offer numerous advantages in terms of promoting uniformity within the oral care industry. Toothpaste viscosity values range from 50,000 to 200,000 cP at a temperature of 20 °C.[61] The toothpaste also has a viscosity of 2000−100,000 (5000−8000) mPa/s at 25 °C.[62] This wide range of toothpaste viscosity values, ranging from 50,000 to 200,000 cP at a temperature of 20 °C and a viscosity of 2000−100,000 (5000−8000) mPa/s at 25 °C, suggests considerable variability in formulation among different toothpaste products. While this diversity may cater to individual preferences, it could have clinical and practical implications. The results obtained from our research completely fall within the defined range and correspond to the values reported in the literature.

These observations have practical implications for the formulation of oral health care products. By understanding the impact of AS on viscosity, manufacturers can customize their products to meet specific consumer requirements. For example, toothpaste formulations can be modified to attain the desired viscosity levels, ensuring both effective cleansing and user contentment. Likewise, the findings are valuable in the development of saliva substitutes or other oral health solutions in which a specific viscosity range is vital for product performance and patient comfort. The viscosity measurements presented in this investigation afford information regarding the rheological behavior of toothpaste, AS, and their mixtures. The results evince the significant influence of AS on the viscosity of the mixtures, underscoring the importance of carefully harmonizing these constituents in oral care product formulations. Furthermore, the consistency of measurements across different viscosimeters bolsters the reliability of the findings, accentuating their relevance in the realm of oral health product development and formulation.

EIS has the capability to provide valuable insights into toothpaste. The utilization of impedance spectroscopy enabled the comparison of the impacts of various toothpaste compositions on the skin and oral mucosa.[63] The investigation revealed that toothpaste containing sodium lauryl sulfate (SLS) elicited positive reactions on both the skin and oral mucosa, whereas toothpaste incorporating betaine did not induce any form of irritation.[64] Moreover, impedance spectroscopy was employed to analyze the process of solidification in dental cements, thereby demonstrating its potential in evaluating the mobility of ions, porosity, and hardening mechanism of dental hydrogel materials.[65] Furthermore, impedance spectroscopy was employed to investigate the characteristics of the oral mucosa, consequently establishing statistically significant variations in impedance indices across different locations within the oral cavity.[66] The impedance data and corresponding phase angle values provide valuable insights into the electrical properties of toothpaste, AS, and their mixtures. Toothpaste possesses essential electrical characteristics that are pertinent to oral care. One study revealed that the toothpaste demonstrated desensitizing, remineralizing, and anticaries properties, as indicated by alterations in the tooth sensitivity index, dental hyperesthesia tests, enamel resistance tests, and dentin hypersensitivity electrical conductivity assessment. These findings underscore the significance of electrical properties in toothpaste for alleviating oral discomfort and fostering oral health.[67] The analysis of impedance and phase angle responses across different frequencies sheds light on the intricate behavior of these substances and their interactions. The impedance measurements disclose a distinct pattern that varies with frequency for both pure toothpaste and its mixtures with AS. Pure toothpaste exhibits a higher impedance at lower frequencies, indicating hindrance to electrical conduction. As the frequency increases, the impedance decreases, suggesting an enhanced electrical conductivity. The addition of AS, especially in equal proportions, significantly reduces impedance across all frequencies, emphasizing the augmentation of electrical conductivity due to the presence of saliva. Moreover, the impedance values for the 33% toothpaste and 67% AS mixtures indicate a moderate improvement in electrical conductivity, achieving a balance between the properties of toothpaste and saliva. Comparative analyses demonstrate that AS alone has impedance values lower than those of pure toothpaste at all frequencies, indicating its lower conductivity.


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However, the presence of various electrolytes in saliva enhances its conductivity compared with a basic saline solution, highlighting the intricate interplay of components in determining electrical properties.

The phase angle data provide further insights, revealing a nuanced interplay between capacitive and inductive behaviors. Pure toothpaste displays a complex pattern of phase angles, indicating a combination of capacitive and inductive responses. The addition of AS suppresses the inductive behavior, shifting the response toward a more capacitive nature, particularly in the 50% toothpaste and 50% saliva mixture. The 33% toothpaste and 67% AS mixture exhibits pronounced inductive behavior, surpassing both pure toothpaste and the 50−50 mixture. Pure AS demonstrates strong inductive characteristics across the entire frequency range, with higher proportions of AS further enhancing these properties.

The statistical analysis confirms significant differences among various samples. The $p$-values below 0.05 for the pairwise comparisons underscore these differences, highlighting the importance of the proportions of toothpaste and AS in determining the electrical properties.

In summary, the impedance and phase angle data elucidate the intricate electrical behaviors of toothpaste, AS, and their mixtures. The addition of AS significantly enhances the electrical conductivity of the mixtures with variations based on the proportions of toothpaste and saliva.

Our principal aim in scrutinizing the EIS modifications postmixing was to evaluate the potential influence of saliva on the performance of toothpaste. Saliva is a multifaceted biological fluid that encompasses numerous constituents capable of interacting with the physical and chemical attributes of toothpaste. By analyzing the impedance values within this dynamic milieu, our intention was to acquire a more comprehensive comprehension of how saliva impacts the electrical conductivity of toothpaste. Moreover, the examination of the EIS changes subsequent to mixing with saliva is crucial for assessing the real-life scenario of toothpaste application. The interaction between toothpaste and saliva is a pivotal facet of oral hygiene practices. Hence, our study sought to provide valuable insights into the practical implications of incorporating toothpaste into daily oral care routines. Furthermore, as emphasized in the literature, the physical and chemical properties of toothpaste, including the concentration and characteristics of solid particles (abrasives), the presence of water-soluble gums (binders), surface active agents, polyol (humectants), and other constituents, can significantly impact its electrical impedance spectrum.[68] By delving into the phenomenon of what occurs subsequent to mixing with saliva, our objective was to explore how these fundamental components contribute to the overall impedance profile and, consequently, the effectiveness of toothpaste.

## 5. CONCLUSIONS

The design of the MF mixer, incorporating intricate serpentine patterns and fabricated with the utilization of advanced SLA 3D printing technology, has been proven to be efficacious in fostering the seamless amalgamation of toothpaste and AS. This exemplary mixer design affords an exceptional degree of precision and control over the flow dynamics of fluids as well as the behavior of particles within the microscale environment.

The behavior of covarine microbeads, which possess a diameter of 200 $\mu$m, within the MF mixer exhibits a commendable degree of predictability. This assertion is firmly substantiated by meticulous simulations and comprehensive experimental validation that unequivocally demonstrate the unfaltering capacity of these particles to traverse through the intricate pathways of the mixer without any deleterious issues pertaining to clogging. Additionally, the covarine microbeads consistently maintained their size and position throughout the course of the fluidic flow process, thereby attesting to their exceptional stability and reliability.

Through the undertaking of viscosity tests, it has been conclusively ascertained that the introduction of AS into toothpaste results in a marked reduction in the overall viscosity of the amalgamated mixture. This salient observation underscores the indisputable fact that the inclusion of AS engenders a heightened level of fluidic behavior, thus auguring well for the prospects of enhanced spreadability and coverage of the toothpaste formulation when applied to the tooth surfaces during the vital process of brushing.

By virtue of the comprehensive utilization of EIS, it has been rendered possible to measure and discern the electrical conductivity of diverse toothpaste and saliva mixtures. Importantly, the addition of AS to toothpaste is unequivocally proven to engender a marked increase in the overall electrical conductivity of the amalgamated mixture. Furthermore, it is crucial to note that the precise proportions of toothpaste and saliva within these mixtures exert a direct influence on the resulting impedance values, thereby underscoring the role that these constituent components play in effecting alterations in the electrical properties of the dental formulations.

Upon undertaking a comparative analysis between the toothpaste−saliva mixtures and their pure counterparts, it becomes readily apparent that the former consistently exhibit noticeably lower impedance values and phase angles. These compelling findings duly corroborate the notion that the inclusion of AS within the mixtures confers a distinct advantage in terms of improved electrical conductivity. Moreover, it merits mention that pure AS manifests distinctive inductive behavior, and its incorporation within these mixtures significantly enhances their overall inductive properties.

The findings of this study bear practical implications for the formulation of toothpaste products. The judicious adjustment of the proportions of toothpaste and AS can serve as a potent tool in the creation of formulations that boast meticulously tailored electrical properties. This, in turn, has the potential to exert a profound impact on the overall effectiveness and efficacy of toothpaste in the realm of dental applications.

In the realm of future research endeavors, it would be highly instructive and illuminating to explore the precise ramifications and consequences of these aforementioned electrical properties on the intricate interplay between toothpaste formulations and the surfaces of teeth. Moreover, a diligent investigation into the potential influence exerted by various additives or compounds on the electrical behavior of dental products holds the promise of yielding invaluable insights that can be leveraged for further advancements and refinements in the domain of product development.

This study makes noteworthy contributions to the field of methodology. Of particular significance is the astute utilization of MF simulations, complemented by experimental validation and electrochemical analysis. These methodologies can be readily extrapolated and applied to the development and characterization of an expansive gamut of diverse dental and medical products, thus conferring a commendable degree of versatility and applicability to the findings of this study.

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M.D., M.V., B.P., S.K., and G.S. conceptualized the work. M.D., M.V., D.M., B.P., S.K., and G.S. designed the experiments, while M.D., M.V., B.P., S.K., A.T., N.F.J., and D.M. contributed to data analysis. M.D., B.P., M.V., D.M., and S.K. conducted the experiments and M.D., M.V., B.P., S.K., A.T., N.F.J., D.M., and F.I. drafted the manuscript. G.S. and F.I. acquired funding and supervised research. All authors revised the manuscript.

### Notes

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