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Viscosity and mixing properties of artificial saliva and four different mouthwashes

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Abstract.
BACKGROUND: Numerous functions of saliva depend on its biophysical properties. Mouth rinses react with saliva and change both their own properties and properties of saliva.
OBJECTIVE: The aim of this study was to define the level of mixing of artificial saliva and mouth rinses, and define their viscosity and its changes at room and body temperature.
METHODS: Artificial saliva, fluoride solutions, chlorhexidine, zinc-hydroxyapatite solution and casein phosphopeptide amorphous calcium phosphate were used. To simulate their mixing, Y-channel PVC chips were used, in two different microfluidics systems. The experiments were recorded with a microscope, then the proportion of mixing was calculated using Matlab. For viscosity measurements rotational viscometer was used.
RESULTS: The results show partial mixing of all solutions with artificial saliva. Measurements with a viscometer indicate different viscosities of all used solutions. Viscosity of a mixture of solution and artificial saliva is always in the range of viscosity of the artificial saliva and the solution separately. Moreover, viscosity of all solutions, as well as mixture with artificial saliva, significantly decreases at higher temperature.
CONCLUSION: Intraoral administration of mouth rinses results in change of biophysical properties of both saliva and mouth rinses. Those changes can affect preventive and therapeutic effect, and therefore oral health.

Keywords: Dissolution, microfluidics, mouth rinses, saliva substitutes

1. Introduction

The effect of the physiological protection of the oral cavity depends on the physical properties of saliva and the change in the rheological properties of saliva has become the subject of increasing research in salivary diagnostics [1–4]. By the mechanism of self-purification, saliva protects against the occurrence

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of the most common oral diseases, caries and periodontitis [5,6]. Decreased salivary secretion or other mouth dryness related complaints are common disorders, affecting 25% of the elderly population, patients using various groups of medications, patients receiving radiotherapy, Sjogren's syndrome and many other conditions [2,7].

Generally, when the symptoms of dry mouth occur, patients are advised to drink large quantities of water, but, water is not able to provide sufficient hydration and lubrication and does not have antimicrobial properties. Saliva preparations or substitutes are recommended as a better solution, since these solutions have a higher viscosity compared to water that is closer to the viscosity of natural saliva [3,8]. Artificial saliva should provide protection of soft oral tissues, enable physiological functions (speaking, chewing, swallowing) and prevent the processes of enamel demineralization. Saliva substitutes usually contain substances of natural origin such as salivary macromolecules including mucins, lysozyme and lactoferrin, but their composition is mainly based on rheological modifiers, electrolytes, preservatives, and sweeteners [3,4,9]. To support a prophylactic effect, management of bacteria in the oral cavity and their effects on periodontal and hard dental tissues, the use of mouth rinses such as chlorhexidine, fluoride solutions and casein phosphopeptide amorphous calcium phosphate CPP-ACP pastes are recommended. As a supplementary tool for the prevention of oral infectious diseases they have become more common in patients suffering from xerostomia [10–12]. At the same time, however, there are recent reports that the use of antiseptic mouthwashes could worsen dry mouth symptoms and that oral care protocols should avoid this iatrogenic practice [13].

Various formulations of artificial saliva are present in the literature and on the market and little guidance is available on the standardization of type of saliva for use in both *in vivo* and *in vitro* protocols [14]. Salivary substitutes expressing acidic behavior have a distinct erosive potential, however most products with higher viscosity exhibit an erosion protective effect. It can be recommended that patients suffering from dry mouth and at high risk for dental erosion should use high-viscosity saliva substitutes and should avoid saliva substitutes with low pH [15].

The oral cavity as a common dissolution and mixing site is often overlooked because of the rapid oral transit since the majority of food, drink and medications are swallowed [5,16]. On the other hand, the oral cavity is unique in that both saliva substitutes and many oral mouth washes that are used daily should exhibit their therapeutic effect in the oral cavity over a longer period of time. All these preparations have different physical properties compared to natural and artificial saliva. As the administration of these preparations is local, i.e. intraoral, they are mixed with saliva and changes in their biophysical properties can considerably alter the therapeutic effect of the antiseptic substances present in the mouth rinses.

The objectives of this research were to determine the viscosities of artificial saliva and four commercially available mouthwashes and the change of viscosity of artificial saliva solution and chemoprophylaxis at different temperatures using two different viscosity measurement systems. Studies were also made of the degree of mixing of artificial saliva and mouthwash using two different microfluidic systems.

2. Materials and methods

In the present investigation, the analyses of carboxymethyl cellulose-based artificial saliva were made according to the prescription of the Belgrade Pharmacy Institution (registered under the Republic of Serbia master preparations), with four mothwashes: 0.1% chlorhexidine solution (Eludril Classic, Pierre Fabre Medicament, Bologna, France); medium concentration (2000 ppm) fluoride solution (Fluorogal Mite,

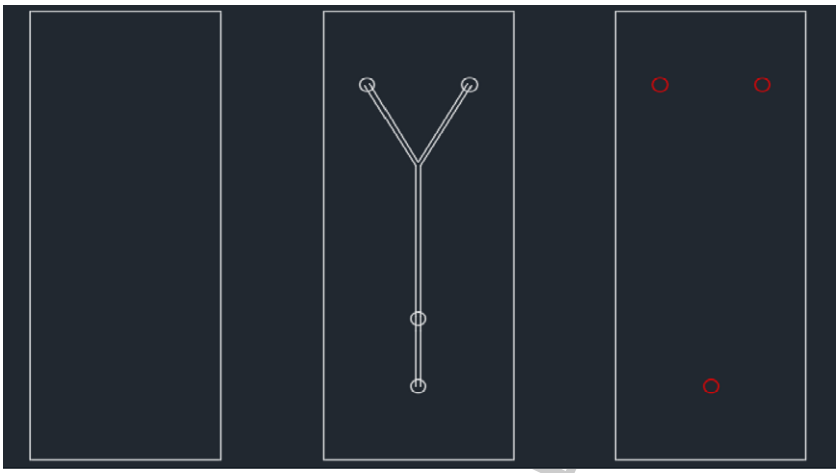

Fig. 1. Design of microfluidic Y-channel PVC chip.

Fig. 2. Experimental setup employing syringe pump.

Galenika, Belgrade, Serbia), zinc-hydroxyapatite (Zahn-Milch, Dr. Kurt Wolff, Bielefeld, Germany) and a casein-phosphopeptide amorphous calcium phosphate (Tooth Mousse, GC America, Alsip, IL, USA).

In order to simulate flow and mixing during the experiment microfluidic PVC chips were used consistently with our previous report [17]. The chip was a Y-channel chip without any obstacles (Fig. 1) with the inlet channels set at a sixty degree angle, with the width of 600 nm and the diameter of both inlets and outlet of 2 mm. The upper and bottom layers of the chip were manufactured by xurography technique, involving the plotter cutter a 125 μm PVC film by a pre-set CAD illustration subsequent lamination. The intermediate layer of the chip was created of a thin layer of laser-cut ceramics.

All experiments were performed using two available systems – the syringe pump (Fig. 2) and Elveflow system (Elvesis) (Fig. 3). Syringe pump setup was prepared as follows: mechanical pressure had been exerted on the syringe with the tested solution resulting in a displacement of a commensurate volume with the applied pressure. Before the experiment started we defined the dimensions (diameter and volume)

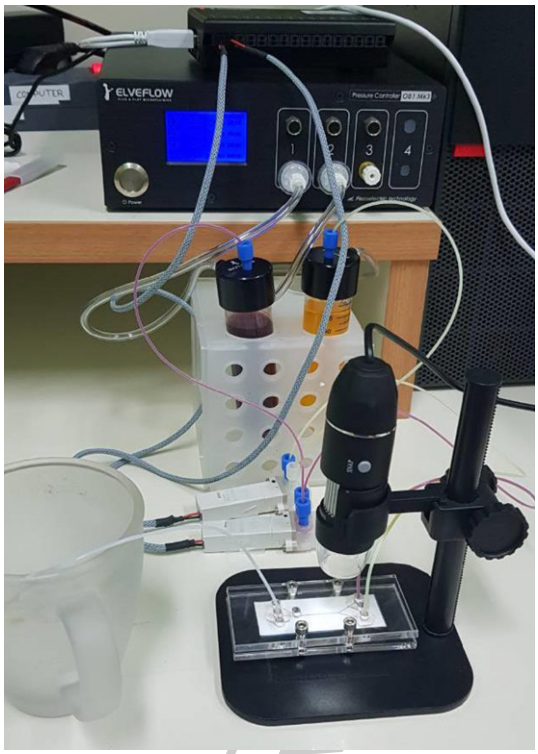

Fig. 3. Experimental system employing pressure controller Elvesys.

of the syringe, while the speed was automatically regulated by adjusting the flow rate of the pump. The Elvesys system works in a very similar way, with little difference – the flow of fluid is made possible by the injection of air by the compressor into the chambers containing the fluid. In this way, the fluid is displaced under equal pressure under which the air is introduced. The main difference between the two methods is the parameter we want to monitor, in the syringe pump experiments we know at all times the exact fluid flow through the chip, while in the experiments using Elveflow system we know the exact pressure. The value of the used pressure matches the flow rate generated with the syringe pump. In the syringe pump experiment, a flow rate of 10 μL/min was used and a pressure of 80 mBar was used on the Elvesys system.

To determine the proportion of solutions mixing, an image processing algorithm in Matlab program was used in consistence with our previous report [17]. The experiments were first recorded with a microscope equipped with camera employing the magnification up to 10X. Artificial saliva was stained with blue color (Food liquid color blue, Eterika doo Trstenik, Serbia) for simpler examination during the process of solutions mixing. The obtained raw images were then processed as follows: for each of the two solutions, the pixel values before liquids mixing and the number of pixels of the observation field were defined. Each color is uniquely determined by three components, red, green and blue (Fig. 4). After mixing, the observational field images were processed by the MATLAB algorithm to define the number of liquid pixels whose values did not change at all during the process of mixing. That was the part that remained unmixed (Fig. 5). Subsequently that value was deducted from the total number of pixels and the quantity portion of the rest, mixed solutions was computed. Because of the thickness, the CPP-ACP paste had been assessed in experimental setups dispersed in 1:3 distilled water.

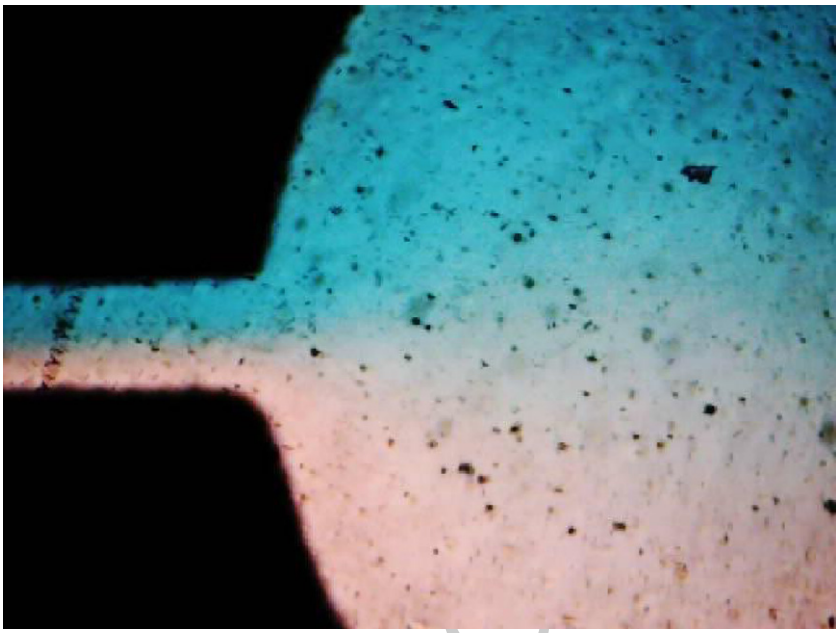

Fig. 4. Visualisation of solutions mixing within the microfluidic chip.

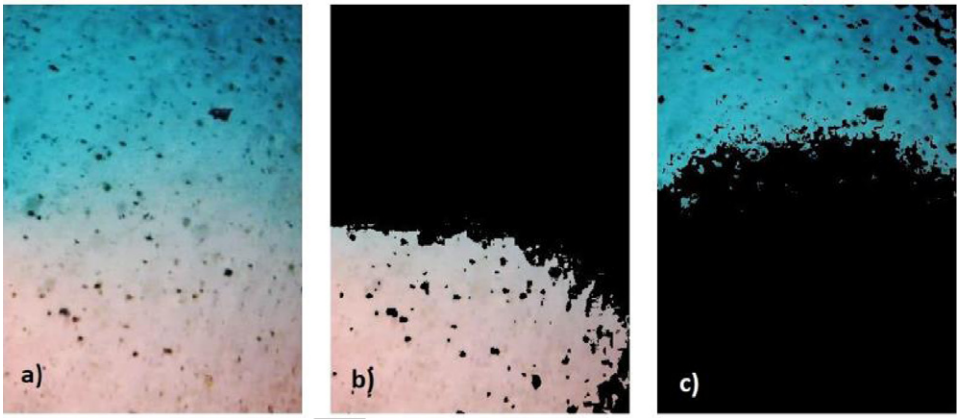

Fig. 5. (a,b,c) Analysis of solution mixing using MATLAB.

The RheolabQC rotary viscosimeter (Anton Paar, Graz, Austria) was used to measure the viscosity of the solution individually as well as the mixture with artificial saliva. Viscosity was measured at two temperatures, room temperature (25 °C) and body temperature (36.6 °C). Two viscosimeter tools were also used, cylindrical (CC27) recommended for viscous solutions and double gap (DG42) recommended for less viscous solutions. Due to its consistency, the CPP-ACP paste could only be tested with a cylindrical tool.

Within the descriptive statistics, the data were presented in the form of mean values and standard deviation. To evaluate how far data were from normality, Shapiro Wilk test was used. At the level of inferential statistics, due to disturbed distribution normality, the significance of the difference between the

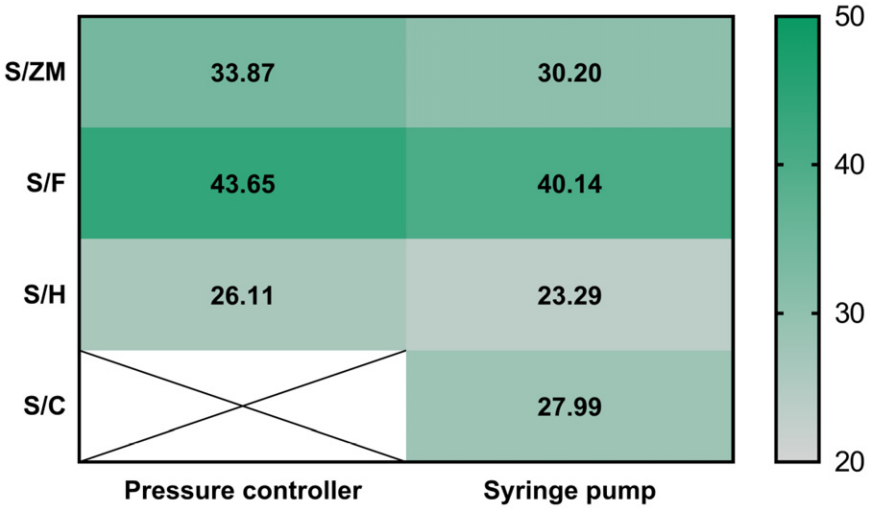

Fig. 6. Heat map showing the proportion of liquid mixing using 2 different experimental setups. S - artificial saliva, C - Casein phosphopeptide-amorphous calcium phosphate (CPP/ACP), H - chlorhexidine solution, F - fluoride solution, ZM - zinc-hydroxyapatite solution.

examined research groups was tested by the Wilcoxon rank-sum test. For comparing multiple treatments (temperature) and devices (viscometer head) a multiple comparison test had been use (Repeated measures ANOVA, non parametric with Durbin Conover pairwise comparison test). The Statistical Package for Social Sciences (SPSS 20.0) was used for all statistical calculations (SPSS nc., Chicago, IL, USA) together with Jamovi software (version 0.9.2.8).

3. Results

The mixing results obtained in Fig. 6 support incomplete mixing of all solutions with artificial saliva, in the range of 23.29% for zinc-hidroxyapatite solutions up to 43.65% for fluoride solutions. Also, the results indicate a higher percentage of mixing using the Elvesys system than the syringe pump.

The results of viscosity measurements on the viscometer are shown in Table 1, Figs 7–9. The obtained results indicate different viscosity of all the test solutions with each other, as well as with the artificial saliva, where the viscosity increases from fluoride solutions (2.44 mPa · s), via chlorhexidine (8.64 mPa · s) and zinc hydroxyapatite solution (26.16 mPa · s), to CPP-ACP paste (7500.43 mPa · s). The viscosity of mixtures of these solutions and artificial saliva are in all measurements within the range of viscosity of the artificial saliva and the solution individually. Also, the viscosity of all solutions as well as the mixture with artificial saliva significantly decreases at higher temperatures. The highest viscosity change at different temperatures was observed with fluoride solutions. An analysis was performed, Wilcoxon rank-sum test comparing the obtained values at two different temperatures (25 °C and 36.6 °C), but also at two different apparatus (cc27 and dg42) and statistically significant differences were presented in the Table 2.

A multiple comparison test (Repeated measures ANOVA, with Durbin Conover pairwise comparison test) revealed statistically significant differences in all pairs of solutions with respect to temperature

Table 1
Mean values (±SD) of evaluated solutions and their mixtures with artificial saliva in two different temperatures using 2 different viscosimeters (values with statistically significant differences are marked with same asterisks) S - artificial saliva, C - Casein phosphopeptide-amorphous calcium phosphate (CPP/ACP), CS - Casein phosphopeptide-amorphous calcium phosphate (CPP/ACP) with artificial saliva, H - chlorhexidine solution, HS - chlorhexidine solution with artificial saliva, F - fluoride solution, FS - fluoride solution with artificial saliva, ZM - zinc-hydroxyapatite solution, ZMS - zinc-hydroxyapatite solution with artificial saliva, CC27 - cylindrical viscosimeter tool, DG42 - double gap viscosimeter tool, /XX - temperature

	ZM CC27/25	ZM DG42/25	ZM CC27/36	ZM DG42/36	ZMS CC27/25	ZMS DG42/25	ZMS CC27/36	ZMS DG42/36
Mean	26.161	25.925	15.124	16.244	12.222	12.510	4.3200	5.2900
SD	3.1256	0.79560	1.9859	0.72208	2.6805	1.4245	1.2601	0.29301
F	F CC27/25	F DG42/25	F CC27/36	F DG42/36	FS CC27/25	FS DG42/25	FS CC27/36	FS DG42/36
Mean	2.4920	1.1651	0.64194	0.70253	3.9889	2.8473	2.2710	1.7831
SD	0.65816	0.24813	0.78124	0.17319	1.1228	0.64210	0.74914	0.39287
H	H CC27/25	H DG42/25	H CC27/36	H DG42/36	HS CC27/25	HS DG42/25	HS CC27/36	HS DG42/36
Mean	9.0965	7.6245	4.7425	5.0298	8.5753	7.0140	4.5649	4.8253
SD	1.3934	0.92256	0.58845	0.83869	1.3723	1.0048	0.85560	0.83833
S	S CC27/25	S DG42/25	S CC27/36	S DG42/36				
Mean	7.5726	5.8901	5.9244	3.6589				
SD	1.2221	0.41206	1.6974	0.37683				
C	C CC27/25		C CC27/36		CS CC27/25		CS CC27/36	
Mean	16201		2995.3		1694.3		12926	
SD	16889		7463.2		1089.4		13004	
Shapiro-Wilk p	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

N = 5 specimens for all solutions, N^1 = 100 measurements per solution.

(25 °C and 36.6 °C) and different apparatus (cc27 and dg42) except for the ZMS solution tested with cc27 and dg42 at the temperature of 25 °C, where the difference was not statistically significant.

4. Discussion

The aim of this experimental study was to determine how the use of chlorhexidine, fluoride solutions and CPP-ACP paste affects the physical properties of artificial saliva, namely viscosity, and therefore the protective effect of artificial saliva, which depends on these properties. In our previous research, a microfluidic system based on a syringe pump only was used and the possibility of mixing human saliva obtained from 5 healthy volunteers with the help of Matlab software was analyzed. In the present investigation, another microfluidic system was included, artificial saliva was used instead of human saliva and the complete set of experiments including viscosity measurements have been performed [17].

It has been observed that it is difficult to select an *in vitro* model to best represent human saliva, in terms of simulating key characteristics and features, and numerous studies had been conducted investigating multiple factors including, pH, buffering capacity and surface tension. In addition, viscosity is analysed

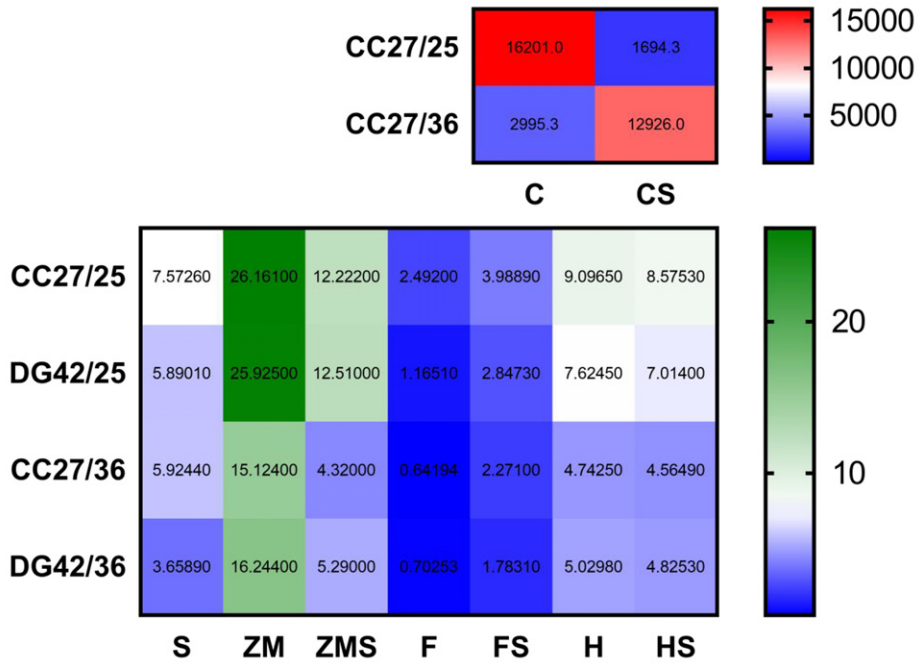

Fig. 7. Heat map showing mean values of viscosities for evaluated solutions and their mixtures with artificial saliva in two different temperatures using 2 different viscosimeters. S - artificial saliva, C - Casein phosphopeptide-amorphous calcium phosphate (CPP/ACP), CS - Casein phosphopeptide-amorphous calcium phosphate (CPP/ACP) with artificial saliva, H - chlorhexidine solution, HS - chlorhexidine solution with artificial saliva, F - fluoride solution, FS - fluoride solution with artificial saliva, ZM - zinc-hydroxyapatite solution, ZMS - zinc-hydroxyapatite solution with artificial saliva, CC27 - cylindrical viscosimeter tool, DG42 - double gap viscosimeter tool, /XX - temperature.

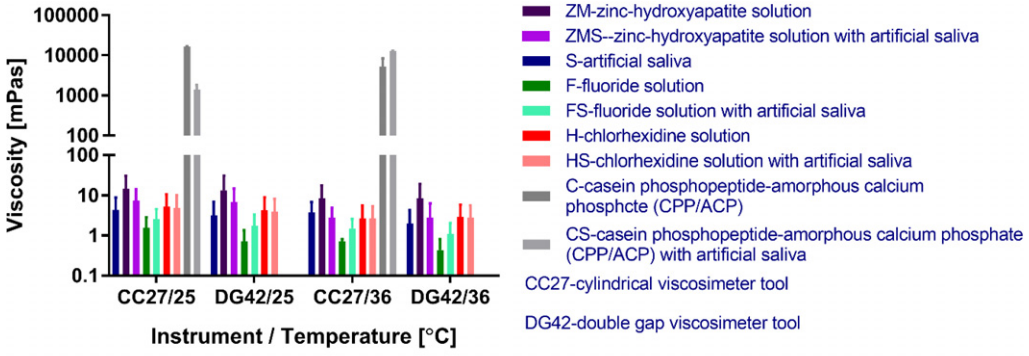

Fig. 8. Histogram showing mean values with standard deviation of evaluated solutions and their mixtures with artificial saliva in two different temperatures using 2 different viscosimeters.

in many studies [18–22]. The present study presents our attempt to extend our knowledge and transfer the salivary diagnostic using microfluidic approach and mathematical models.

The viscosity of human saliva, both stimulated and unstimulated had been extensively analysed in the literature, and the reported values range from 1.5 mPa · s over shear rate of 1–300 s^{-1}, over 3–8 mPa · s

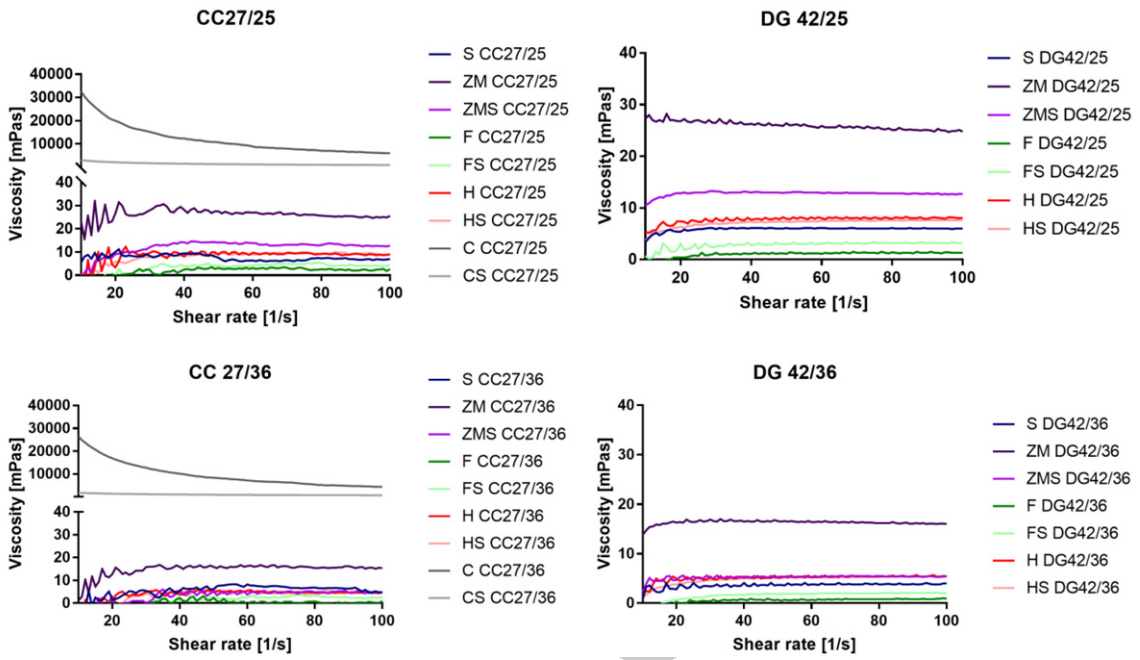

Fig. 9. The viscosity of investigated solutions in shear rate range up to 100 s (−1) using CC27 measuring system (upper and lower left) and DG 42 (upper and lower right) on 25 °C (up) and 36 °C (down).

at a shear rate of 90 s^{-1}, up to 100 mPa · s at a shear rate of 0.02 s^{-1} [16]. Research groups used different shear rates, temperatures and types of rheometer and often small sample sizes. This research aims to address these issues by using physiological temperature and assessing viscosity across a range of shear rates anticipated to occur during the action of mouthrinsing. The results from the present investigation obtained for artificial saliva, whose viscosity ranged from 3.6 mPa · s to 7.57 mPa · s are completely comparable with the viscosity of human saliva reported by Rantonen and coworkers [20].

Viscosity is the resistance by which individual layers of fluid resist the movement of one relative to the other, that is, a type of internal friction that causes fluid to flow at a constant rate. Flow resistance is the result of several factors, including intermolecular interactions, shape and size of molecules [2]. Rheology deals with the deformation of materials exposed to stresses or forces. Newtonian viscous fluids are defined by the property that stress is linearly related to load rate. These models adequately represent the behavior of different materials, such as metals, air, water, and oils, for a wide variety of movements [3]. However, there is a large group of materials and biomaterials, such as saliva, that cannot be described by a simple elastic or viscous rheological model. In fact, they exhibit both viscous and elastic properties, and there are no mathematical functions to fully explain their behavior during the demanding and complex movements to which they are exposed.

The rheological properties of saliva result from many factors and it has shown to behave as a non-Newtonian fluid whose viscosity decreases with increasing shear rate [9]. Since natural saliva is a non-Newtonian fluid, its viscosity varies with the shear rate. The viscosity of saliva at rest and when the shear rate is in the range of 0.1–1/s is much higher than the viscosity during chewing and speech when the shear rate is about 60 and 160 1/s [4]. There are recommendations in the literature that higher values of shear rate (as for chewing is 160 s (−1)) should be tested [21]. In defining the range, our intention was

Table 2
Wilcoxon rank-sum test

		95% Confidence interval			
		Statistic	*P*	Lower	Upper
ZM CC27/25	ZM DG42/25	3212.00	<.001	0.40007	0.85000
ZM CC27/25	ZM CC27/36	4005.00	<.001	10.50000	11.20008
ZM CC27/25	ZMS CC27/25	4095.00	<.001	13.19999	13.84995
ZM DG42/25	ZM DG42/36	4371.00	<.001	9.45000	9.75008
ZMS CC27/25	ZMS DG42/25	1954.00	0.581	−0.54997	0.35000
ZMS CC27/36	ZMS DG42/36	106.00	<.001	−1.00007	−0.60002
F CC27/25	F DG42/25	2763.00	<.001	1.29993	1.54997
F CC27/25	F CC27/36	1875.50	<.001	1.70002	2.40008
F CC27/25	F CC27/36	1875.50	<.001	1.70002	2.40008
F DG42/25	F DG42/36	3079.00	<.001	0.44999	0.49997
FSCC27/25	FSCC27/36	2329.00	<.001	1.54999	1.94997
FS DG42/25	FS DG42/36	3478.50	<.001	1.00001	1.10007
H CC27/25	H CC27/36	3233.00	<.001	4.34997	4.60006
H CC27/25	H DG42/25	3581.00	<.001	1.24995	1.60004
H CC27/25	H CC27/36	3233.00	<.001	4.34997	4.60006
H DG42/25	H DG42/36	4465.00	<.001	2.59998	2.65001
HS DG42/25	HS CC27/36	3003.00	<.001	2.19999	2.54995
HS DG42/25	HS DG42/36	4186.00	<.001	2.19995	2.25000
S CC27/25	S DG42/25	4084.50	<.001	1.35003	1.90002
S CC27/25	S CC27/36	3112.50	<.001	1.09999	2.14998
S DG42/36	S CC27/36	121.50	<.001	−2.75002	−2.10003
C CC27/25	CS CC27/25	5050.00	<.001	9124.44995	12454.60004
C CC27/25	C CC27/36	5050.00	<.001	9247.04996	12282.45003
CS CC27/25	CS CC27/36	0.00	<.001	−10074.05000	−7106.99999

to mimic real clinical situation as much as possible, following the presumption that during the action of mouth rinsing anticipated values should be substantially lower compared to chewing. We followed the reports from the literature that state that, the exact magnitude of the applied shear rates in the mouth is not known; estimates range from 0.1 or 10 to 1000/s [22]. In addition it has been reported that shear rates appear to be modulated by the substance texture and they changed during oral processing [23]. Also it has been pointed to the fact that probably a wide range of shear rates are involved, and different shear rates may be present simultaneously in the mouth, e.g., the bite pouched in the cheek is at a shear rate of zero [24]. Finally in the report published by Bonda et co-workers [25] the majority research had been summarized and in the majority of the studies shear rates did not exceed 100/s, making our findings applicable for possible data comparison.

There is no uniform information in the literature about the role of viscosity of the mouth rinses in their erosive potential. Saliva substitutes and mouthwashes enter the complex processes present in the oral cavity, forming specific connections at the mucosa–liquid and enamel–liquid interfaces. As a result, more viscous agents can affect the ion exchange rate at the enamel–liquid interface, compromising both demineralization and remineralization processes, which should not be expected from the solutions whose main task is to promote remineralization and decrease the extent of demineralisation [26]. And the recent clinical reports go in line with that, suggesting that increasing the viscosity of the mouthwash solutions reduced enamel loss by erosion; however, this effect was small and only observed when the solutions were

applied only once a day. There are attempts to neutralize the erosive potential of commercially available mouthwashes with adding of various viscosity increasing substances [27].

Chlorhexidine digluconate is one of the most commonly used disinfectant in dentistry, which in lower concentrations acts bacteriostatically and in higher has bactericidal effect. It also has a fungicidal effect. As a mouthwash it has numerous indications for its administration, ranging from preventative plaque control, through preoperative and postoperative disinfection of the oral cavity, to the treatment of gingivitis and periodontopathy [5,28]. In the present investigation chlorhexidine solution exhibited the lowest proportion of the mixing, while the range of viscosity was similar to the artificial saliva. The reported pH values of the chlorhexidine solutions vary from 5.50 to 6.88 [29]. It has been recommended in the treatment of xerostomia [30]. but also there are concerns about its effectiveness in dry mouth symptoms management [13]. Our data regarding the viscosity of mixture (chlorhexidine/artificial saliva) at 36 °C, that was lower compared to artificial saliva itself go in line with the concerns about the recommendations of chlorhexidine usage for dry mouth management, since we could expect synergistic effect of lower viscosity, acidity and lack of remineralisation properties.

Fluorides are known to be a potent anticariogenic agent because they have shown the inhibition of demineralization and force remineralization processes on the tooth surface. Sodium fluoride rinse solutions are widely used today for the prevention of caries. A study conducted by Cretescu et al. states that the amount of fluoride in antiseptic solutions significantly affects physicochemical properties of these solutions, including viscosity [31]. For solutions with different concentrations of fluoride, they state a viscosity range of 1.4–2.01, values that completely correspond to the results obtained in our study. Of particular importance is the fact that precisely with the lowest viscosity solutions tested in the mentioned study, the lowest pH values of 5.28 are recorded, which indicates the significant erosive potential of the solution of such properties [31]. In the present investigation, the lowest values of viscosity parameters were recorded for fluoride samples, while the mixing proportion was the highest. The literature has already shown that increasing the viscosity of fluoride solutions leads to a reduction of enamel erosion, completely in line with the recent attempts to improve the efficacy of fluoride solutions by viscosity modulations [32]. Viscosity is controlled by internal friction forces between adjacent layers of fluid. Viscosity was measured at room temperature of 26 °C in the majority of available literature sources. Given that the human body temperature is around 37 °C, in our study all viscosities were measured at two temperatures, 26 °C and 36.6 °C, respectively. The results of our study show that the hierarchy of absolute viscosity values remains preserved with increasing temperature, and uniform fluctuations in viscosity at higher temperatures are observed at all tested fluids and their solutions. All chemoprophylactic agents analyzed in this study have lower viscosity values at physiological temperatures.

Milk-based pastes containing casein-phosphopeptide (CPP) and amorphous calcium phosphate (ACP) nanocomplexes have also been used. These pastes have shown they reduce demineralization and force remineralization of hard dental tissues [33,34]. There are reports in the literature about their efficient use in dry mouth symptoms management [11]. The results from the present investigation go in line with these recommendations since these high viscous and alkaline preparations do not pose a risk, at least regarding the erosive attack.

Hydroxyapatite based preparations offer a promising option for oral care in persons with reduced salivary flow [35]. There are reports in the literature confirming its remineralisation potential based on hydroxyapatite microclusters reducing initial bacterial adhesion to enamel considerably, suggesting sensibly supplement current approaches in dental prophylaxis based on fluoride [36]. The results in the present investigation showed the major decrease in viscosity after mixing with artificial saliva, a parameter that definitely requires further elucidation.

Our work suggests that the potential of mouthwash erosion and enamel wear requires further examination, both *in vitro* and *in vivo*, as some physicochemical factors, such as salivary flow and buffering capacity, may influence their overall impact on the dental enamel [27,32].

In the present study 2 microfluidic setups were used to simulate mixing of artificial saliva and different mouthwashes. Microfluidics has been developed quickly in last two decades offering countless applications in biomedical research. This so called microfluidics revolution arose due to the some advantages offered by system miniaturization, the high analytical throughput, enhanced sensitivity and improved analytical performance [37]. When it comes to salivary *in vitro* investigation its applicability has been definitely confirmed.

Data acquisition and analysis in salivary *in vitro* experiments frequently employ image processing tools such as Matlab [38,39]. Image segmentation is state-of-art method widely used in many different branches of research today. It is efficient, quick and primarily low cost way to extract needed information. In this present investigation, rate of mixed and unmixed fluids could be unequivocally determined under certain conditions. Conditions that needed to be fulfilled were that source of light around microfluidic chip and position of camera were constant and unchanged throughout whole experiment. Having this provided, it was enough to film each fluid separately, before conducting an experiment and then to film experiment itself with enough images. From images where fluids were separated, ranges of pixel values could easily be determined by taking minimum and maximum value for each of three channels of color (red, green and blue).

The results of the research show that there is no linear, defined and predictable change in the viscosity of saliva substitutes and local antiseptic solutions, and that the degree of mixing depends on both the flow of liquid substances and the pressures to which liquids are exposed. Viscosity changes and the degree of solution mixing can significantly affect the properties of saliva, as well as the physicochemical properties of the active substances in antiseptic solutions, thereby modifying the expected preventive ones, prophylactic and therapeutic effect.

Local intraoral administration of various mouthwashes results in incomplete mixing with artificial saliva. Mixing them changes the originally measured viscosities of both saliva and the mouthwash itself. Also, at the temperature of the body, which governs that in the oral cavity, the viscosity of both saliva and artificial saliva and their mixtures decreases significantly. These changes can have a significant impact on the effects of saliva and solution, and therefore on oral health.

Conflict of interests

The authors declare no conflict of interest.

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