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Variability of Saliva Viscosity - Potential Impact

Lara Eltze, Maren Eltze and Antonio Garcia

Abstract

As novel COVID-19 testing develops, saliva has become of increasing interest as an alternate biological sample for rapid testing. The appeal in saliva-based testing lies within the ease of which samples are collected, as well as patient comfort throughout the collection process. With this, it has become increasingly important to delineate the characteristics of saliva viscosity due to its effects on the movement and interactions of the substances and molecules found within it. The characteristics that affect saliva viscosity include the presence of aggregates, variations in temperature, and time elapsed between sample collection and testing. Understanding how physicochemical properties and temperature affect saliva's viscosity are important in generating guidelines for proper sample handling in saliva testing to ensure consistent and reliable results. In this study, passive sampling of saliva was analyzed. This type of collection ensures a more uniform saliva composition, suggesting that variations in viscosity can be attributed solely to modifications in saliva handling post-collection. The data suggested that saliva viscosity is greatest immediately following collection of the saliva sample, increases with higher quantities of aggregates in saliva, and decreases tremendously when the sample has been frozen and thawed to room temperature. These findings suggest that to ensure accuracy and uniformity in quantitative saliva-based test results, protocols should favor the testing of a sample immediately following its collection. The implications of these results in optimizing saliva testing are far reaching. The value of saliva based testing extends far beyond COVID-19 or other disease testing. It is also gaining utility in understanding daily fluctuations in hydration state and in other wellness applications.

Keywords: saliva, viscosity, point-of-care, diagnostics, Cannon-Fenske, viscometer

1. Introduction

As novel COVID-19 testing develops, saliva has become of increasing interest as an alternate biological sample for rapid testing [1]. The appeal in saliva-based testing lies within the ease of which samples are collected, as well as patient comfort throughout the collection process [2]. Yacoubian Jr., Wish, and Perez (2001) found that the benefits in the ease of saliva collection were multifaceted. These benefits include the uncomplicated nature of collection, which, coupled with a low risk of direct contact and contamination, makes salivary diagnostics an attractive alternative to biological sample collection where contamination may be more challenging to avoid, such as with blood or urine analyses. For these reasons, saliva-based testing has become an increasingly popular choice in the creation of novel forms of

diagnostic testing. With this, it has become increasingly important to delineate the characteristics of saliva viscosity due to its effects on the movement and interactions of the substances and molecules found within it. In the context of this study, viscosity refers to internal friction of a fluid, which is marked by the resistance of a fluid to flow [3].

While viscosity can affect the interactions and molecules within saliva is important to note in developing diagnostic tests, salivary viscosity itself can also be seen as an important factor in maintaining oral and overall health. A study by Katsuhiko Kitada and Takahiko Oho (2011) found that an increase in saliva viscosity decreases the bacterial co-aggregation between *Streptococcus oralis* and *Actinomyces naeslundii* [4]. Under normal circumstances, co-aggregation can prevent bacterial infection in the oral cavity, as co-aggregated bacteria may be swallowed before forming attachments within the oral cavity. The study indicated that increasing saliva viscosity decreased formation of these co-aggregated bacteria, which may allow for further health problems, such as pneumonia or other infections that may be brought on by the aspiration of oral bacteria or microorganisms [4]. The demonstrated health implications surrounding salivary viscosity further suggests the importance of developing protocols to accurately measure salivary viscosity following saliva collection.

The characteristics of salivary viscosity, namely the presence of aggregates, variations in temperature, sample handling, and time elapsed between sample collection and testing, serve as points of interest in the creation of laboratory protocols for salivary-based rapid diagnostic testing. Understanding how external factors affect saliva viscosity are important in generating guidelines for proper sample handling in saliva testing to ensure consistent and reliable results.

Multiple studies demonstrated in the literature reflect the variability of saliva viscosity. The 1998 Rantonen and Meurman study concluded that salivary viscosity can be dependent on the method of its production. Particularly, whether secreted by the submandibular, sublingual, or palatal glands [5]. Although the study demonstrated that the quantity of mucin within each saliva sample of differing origin did not change, the species of mucin did. Particularly, it was demonstrated that the saliva stemming from the sublingual glands demonstrated more elasticity than those of the submandibular and palatal glands, which would affect the viscosity of the saliva. In addition, the 2016 study by Antoon Ligtenberg, Erwin Liem, Henk Brand, and Enno Veerman found that acute exercise correlated with a significant increase of saliva viscosity when collected shortly thereafter [6]. These findings were parallel with the Rodica Murineanu, Corina Stefanescu, Agripina Zaharia, Carolina Davidescu, and Sorin Popsor (2011) study that found medication, general illness, and acrylic dentures to all correlate with a change in saliva viscosity [7]. This study suggested medication and disease state may affect saliva viscosity. For example, complete acrylic dentures were specifically found to correlate with an increase in salivary viscosity. It is also interesting to note the apparent correlation between salivary viscosity and dental cavities. A 2014 study by Animireddy et al found that in a sample of 75 school children, the cavity-free children had on average higher salivary viscosity than their counterparts [8]. These findings delineate some of the known variability to saliva viscosity discussed in the literature, which further demonstrate the necessity of qualifying the properties and behavior of saliva viscosity.

Beyond the variability of salivary viscosity, the level of normal viscosity is very different from that of other commonly used human biofluids in diagnostic testing. This is an important factor to note in the development of such tests, especially when considering technologies previously developed for other biofluids. The viscosity of normal cerebrospinal fluid, for example, is remarkably close to that of water, which is 1.00 cSt at 20°C [9, 10] Similarly, the kinematic viscosity of urine is 1.07 cSt at the

same temperature [11]. These examples are lower than the kinematic viscosity of normal blood, which is around 3.65 cSt at 21.2°C [12]. While there is variability within the viscosities of these human biofluids, they are far lower than what we expect of human saliva, an important challenge to overcome in developing diagnostic testing.

Due to the interest in point-of-care saliva-based diagnostic testing, and based on the current literature demonstrating potential variabilities in saliva viscosity and associated causes, it is rather surprising that the literature on salivary viscosity characterization for protocol creation is rather sparse. This study hopes to address some of the gaps in the literature pertaining to salivary properties by exploring how viscosity changes upon freezing and subsequent thawing, and how it changes over time with consecutive trials, using the Cannon-Fenske experimental protocol, with the goal of aiding in the development of laboratory protocols pertaining to salivary-based diagnostic testing.

Based on the previous literature at hand, the research questions of this study are as follows:

How does the viscosity of collected saliva change over time with subsequent trials? How does the viscosity of collected saliva change after freezing and subsequent thawing?

2. Application of research

In the absence of detailed information in the research literature, this study seeks to better understand the specific properties of saliva viscosity, and how saliva viscosity reacted to factors that are integral in the creation of lab protocols; specifically, how the samples are stored. It is not uncommon for biological samples to be frozen or cooled, and this makes sense with respect to slowing down bacterial contamination and maintaining biological molecules of interest. It is well understood that many human proteins, enzymes, vitamins, degrade over time [9] and that the degradation over time can be diminished by freezing or cooling samples beyond given temperature degradation thresholds, to allow for long-term storage.

Changes within the aggregates commonly found in human saliva, such as mucins, may also be affected by temperature and shear forces. Enzymes, such as salivary alpha-amylase, and hormones, such as cortisol, will degrade over time unless this process is inhibited, typically by freezing samples to -20 degrees Celsius, or below [10]. However, it is important to determine the effects of sub zero temperatures on viscosity itself, as the viscosity of the saliva may be impactful in how the aggregates are measured via point-of-care salivary biosensors. This was reflected in the 2013 Robles et al. study which found that the most consistent and reliable salivary alpha-amylase biosensor data was obtained from frozen and centrifuged passive saliva samples, rather than samples that were collected as fresh, passive, drool [11]. The authors hypothesize this discrepancy to be due to various factors of the saliva itself (such as mucin molecules), which may interfere with the device in question by preventing close binding to the sensor surface, as it attempts to detect quantities of salivary alpha-amylase. Also, this hypothesis reflects the prediction that salivary viscosity is an important factor in molecular measurements. While the aggregation effect was disadvantageous when in reference to the assessing quantities of the enzyme salivary alpha-amylase, it may actually be preferable when measuring quantities of different gases within saliva samples. This further delineates the importance of taking a closer look at physical salivary properties, in order to approach proposed handling methodologies appropriately, depending on the given purpose of the saliva sampling.

3. Protocols

As previously mentioned, we hoped to better understand the properties of saliva viscosity in regards to different methodologies in sample preservation or usage. For this reason, two cycles of laboratory trials were conducted, with the aim of determining how saliva viscosity was affected. The first study aim is to determine how time alone affects saliva samples. The second trial aims at determining whether freezing and subsequent thawing affect the viscosity of the sample, as well. Both phases of data collection were done at a Biomedical Engineering laboratory, at Arizona State University, Tempe, and human saliva samples were collected within this department.

Participants were instructed to not eat within an hour of sample collection, and were then asked to drink approximately 100 mL of water immediately prior to saliva collection. This was to prevent short-term dehydration effects from confounding our variables. In addition, this aided in the ease of saliva collection. Participants were then asked to collect approximately 15 mL of passive saliva over a span of 25 minutes, into a plastic vial. The goal for saliva collection included diminishing the amount of air bubbles trapped within the saliva, by collecting the saliva very carefully, slowly, and with as little movement as possible. Foam-like saliva that is saturated with small air bubbles was not included in the overall 15 mL amount of passive saliva collected.

The viscosity of the collected saliva sample was measured using a Size 350 Cannon-Fenske apparatus, with a capillary radius of 0.045 cm, a shear rate of 2.08 1/s at 10cSt, and a viscometer constant of 0.5 cSt/s, which was cleaned and dried prior to commencing the viscosity protocol. The saliva sample was then poured into the apparatus, and allowed to flow through, while efflux time was measured concomitantly, indicating the time required for the meniscus of the viscous fluid to flow between the designated markings. This viscometer procedure was replicated 10 times consecutively for each collected sample, after which each sample was frozen and subsequently thawed the following day, at which point the viscometer procedure was performed again. A series of viscosity measurements of a 50% glycerol/water control solution was tested in the same manner to act as a control variable.

The kinematic viscosity of each trial was calculated using the efflux time and viscosity constant in the following relationship:

$$\text{Kinematic Viscosity} = (\text{viscometer constant}) \times (\text{efflux time (s)})$$

This procedure reflected the first half of the research questions, as to how saliva viscosity changes with time [12]. By comparing the time elapsed for the viscosity of human saliva with the glycerol/water solution, we are able to visualize how viscosity properties may be affected by the presence of the aggregates in unprocessed human saliva, which are not present in the glycerol/water mixture.

4. Results

Data was collected for each measurement of salivary viscosity. The initial graph (**Figure 1**) represents the methodology behind the first research question; how does salivary viscosity behave over time? **Figure 2** represents the viscosity of saliva (as average kinematic viscosity), with freshly collected samples of saliva, compared to with samples that were frozen, and subsequently, thawed for analysis.

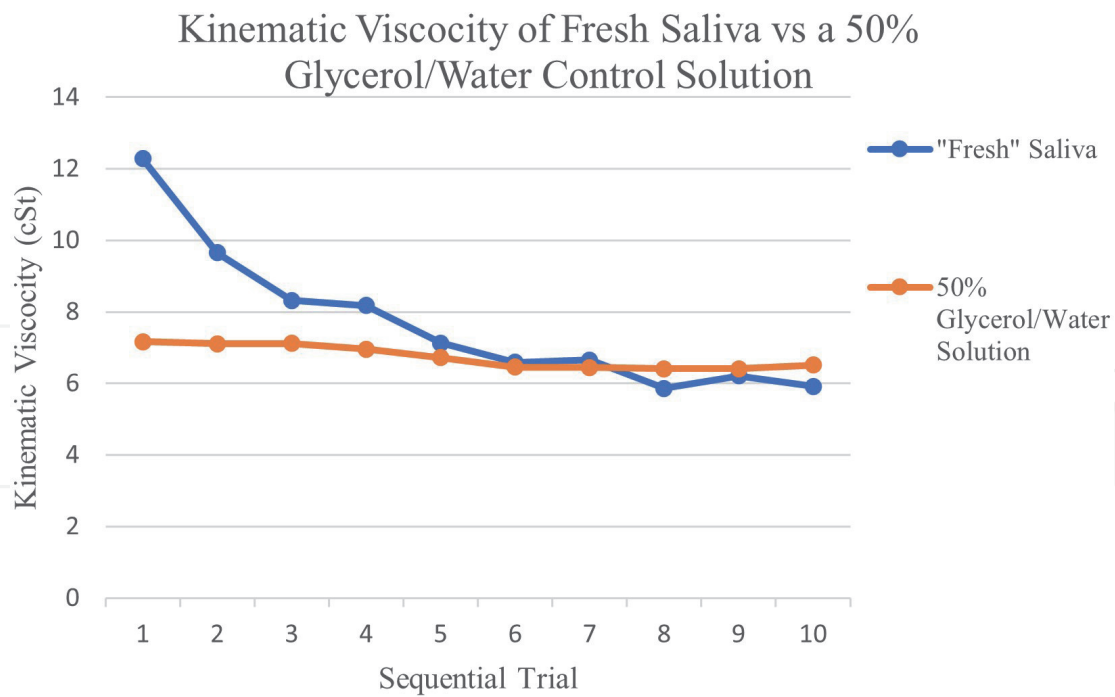


Figure 1.
 This figure evaluates how salivary viscosity changes with time over subsequent trials following collection. This arbitrary time is demonstrated in sequential trials, as trials were completed one after the other following collection. This saliva kinematic viscosity is contrasted with that of a 50% glycerol water solution, acting as a control. In the passive saliva trials, we see a stark decrease in kinematic viscosity with each subsequent trial, however, the glycerol/water solution shows a slight variation within an expected range given the experimental apparatus and simple laboratory control of room temperature (22 C).

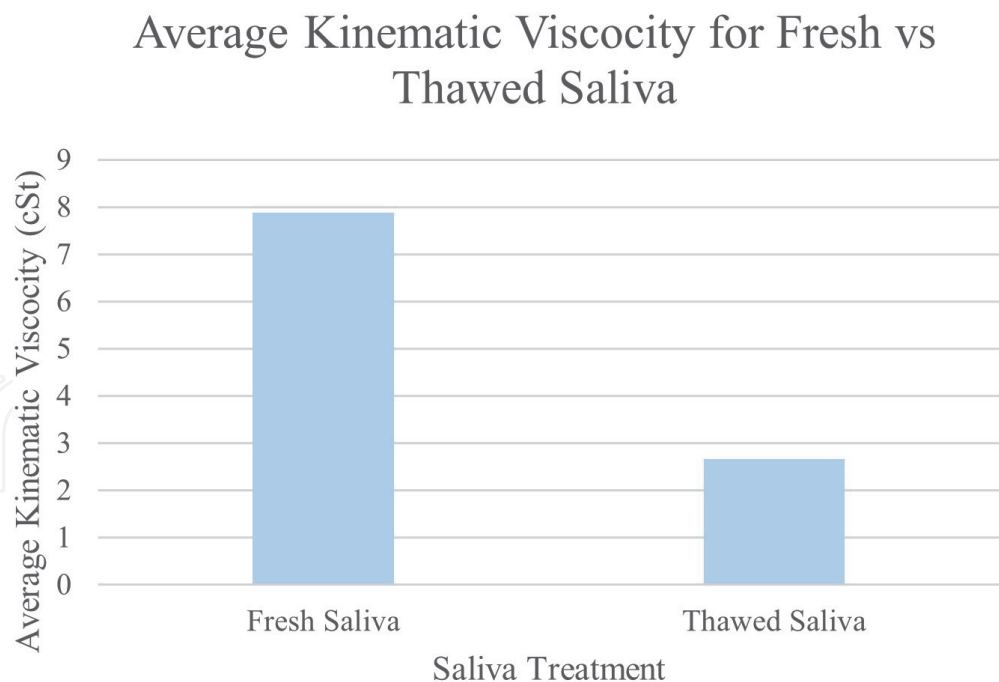


Figure 2.
 This figure demonstrates the statistically significant discrepancy between fresh and thawed passive saliva samples at 22 C. each bar represents an average of 10 trials. One outlier was removed from the thawed trial group as it was handled outside the guidelines of the lab protocol.

5. Discussion

There are several points of interest with regards to the conclusions drawn from the experimental design that reflect the difference in how fresh saliva behaves,

compared to fresh saliva, and compared to saliva that had been frozen and re-thawed. Such comparisons are reflected in the literature by the 2019 study by Johannsen et al. which found that salivary viscosity measurements varied depending on whether the saliva was untreated or subject to magnet-beating prior to viscosity measurements at low shear rates [13].

One aspect that is of interest is whether there is a methodology that can guide how fresh saliva can be processed in order to have a consistent flow property or ensure that the major aggregates, presumably due to entangled mucin chains, can be minimized quickly so that a rapid test can be performed (**Figure 3**).

Bansil et al. [14] provide a molecular interpretation on how the structure of mucin leads to entanglements among the biopolymers in solution and create a range of viscosity effects depending upon concentration and mucin type. They suggest that a dilute solution of mucin generally has viscoelastic behavior that depends upon shear rate. A more general approach to the viscosity behavior of biopolymer solutions is given by Picout and Ross-Murphy [15] who provide experimental verification of the Cross equation:

$$\eta = \eta_{\infty} + (\eta_0 - \eta_{\infty})/[1 + (\lambda^m \gamma^m)]$$

with lambda being a time constant and gamma the shear rate.

Comparing the Cross equation to the data shown in this study in **Figure 2** suggests that repeated shearing with a Cannon-Fenske viscometer is a cumulative effect, so one may conjecture the following modified forms of the Cross equation could explain the apparent viscosity change in saliva after repeated shearing due to the capillary flow within the viscometer.

$$\eta = \eta_{\infty} + (\eta_0 - \eta_{\infty})/\sum_{n=0}^k [1 + k (\lambda^m \gamma^m)]$$

or

$$\eta = \eta_{\infty} + (\eta_0 - \eta_{\infty})/\{[1 + (\lambda^m \gamma^m)T]\}$$

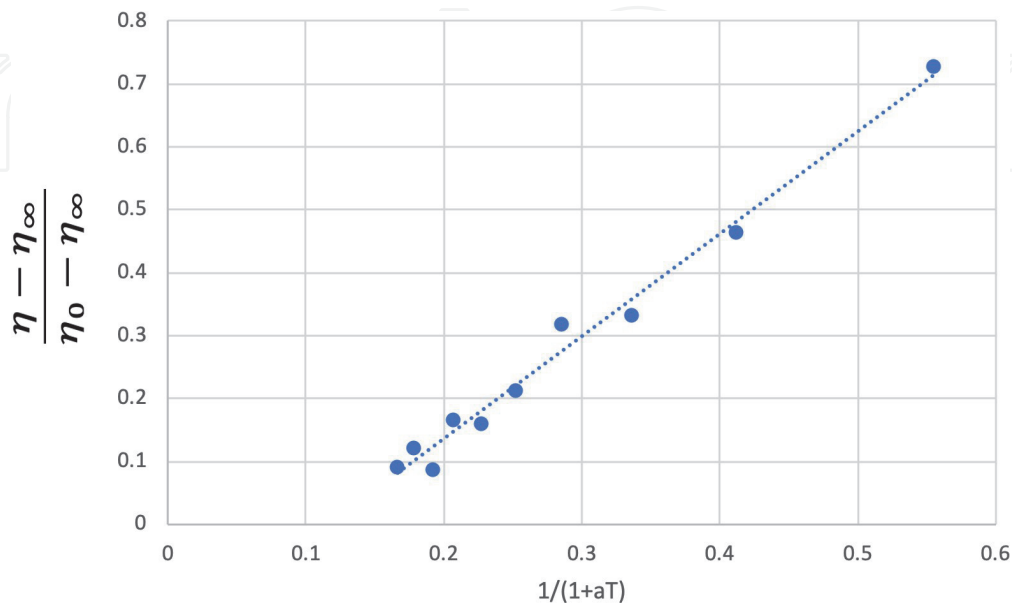


Figure 3. This figure demonstrates the cross equation fit, using the limits of η_{∞} and η_0 . The independent variable is demonstrated to be $1/(1 + aT)$.

where n is an integer and k is the number of repeat measurements in the first equation and T is the total elapsed time of shear for the combined repeat measurements. It is not as important to determine which equation may be better at predicting data than to understand how to use the concept to prepare saliva samples in a variety of ways rather than relying solely on freezing or centrifugation which can be cumbersome and time consuming.

The potential utility of these modified Cross equations is in determining a way to rapidly shear saliva samples using a simple microfluidic device or mixer, rather than subjecting the saliva to freezing or flowing the saliva through a longer tube to simulate the cumulative shear thinning shown in **Figure 2**. The shear rate experienced in the Cannon Fenske viscometer used for these experiments is on the order of 2 s^{-1} , so based on the **Figure 2** data of approximately a total time of 80 seconds of shearing is needed in order to reach a stable and minimum kinematic viscosity a device capable of deliver a shear rate of 200 s^{-1} which is well within the reach of portable and low cost homogenizers [16–19].

6. Conclusions

Salivary viscosity can be an important parameter to consider when designing diagnostic devices for rapid testing. Consideration should be given to the fact that not only is saliva viscoelastic, but its apparent viscosity can change by mild shearing over a period of time. Shearing at rates as low as 2 s^{-1} can decrease its kinematic viscosity by more than half, which could change some kinetics of enzyme action, sensor signal development, or diffusive transport. After approximately a total time of 80 seconds of shearing at 2 s^{-1} can lead to a stable and minimum kinematic viscosity. The concept of biopolymer viscosity behavior being modeled by the Cross equation suggests that a device capable of delivering shear rates of 200 s^{-1} and above may be able to modify the mucin superstructure sufficiently to provide saliva samples with consistent apparent viscosities. Microfluidic devices or low cost hand-held homogenizers could very quickly deliver the needed shearing action in order to provide a more consistent saliva sample, in terms of its viscous properties.

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Conflict of interest

The authors declare no conflict of interest.

Notes/Thanks/Other declarations

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References

- [1] Khurshid Z, Asiri FYI, Al Wadaani H. Human saliva: Non-invasive fluid for detecting novel coronavirus (2019-nCoV). *International Journal of Environmental Research and Public Health*. 2020;17(7):2225
- [2] Speckl I, Hallbach J, Guder W, Meyer L, Zilker T. Opiate Detection in Saliva and Urine—A Prospective Comparison by Gas Chromatography–Mass Spectrometry. *Journal of Toxicology – Clinical Toxicology* [Internet]. 1999 Jun [cited 2020 Jul 14];37(4):441. Available from: <https://search.ebscohost.com.ezproxy1.lib.asu.edu/login.aspx?direct=true&db=aph&AN=7430260&site=ehost-live>
- [3] Cleveland CJ, Morris C. *Dictionary of Energy*. 2nd ed. Elsevier Science & Technology; 2014
- [4] Kitada K, Oho T. Effect of saliva viscosity on the co-aggregation between oral streptococci and *Actinomyces naeslundii*. *Gerodontology*. 2011 Nov 13; 29(2):e981-e987
- [5] Rantonen PJ, Meurman JH. Viscosity of whole saliva. *Acta Odontologica Scandinavica*. 1998;56(4):210-214
- [6] Ligtenberg AJM, Liem EHS, Brand HS, Veerman ECI. The Effect of Exercise on Salivary Viscosity [Internet]. MDPI. Multidisciplinary Digital Publishing Institute; 2016 [cited 2020Jul25]. Available from: <https://www.mdpi.com/2075-4418/6/4/40/html>
- [7] *Romanian Journal of Oral Rehabilitation*. [cited 2020Jul25]. Available from: <http://www.rjor.ro/evaluation-of-total-unstimulated-saliva-viscosity-in-complete-edentulous-patients/?lang=ro>
- [8] Animireddy D, Reddy Bekkem VT, Vallala P, Kotha SB, Ankireddy S, Mohammad N. Evaluation of pH, buffering capacity, viscosity and flow rate levels of saliva in caries-free, minimal caries and nursing caries children: An in vivo study. *Contemporary Clinical Dentistry*. 2014; 5(3):324-328. DOI: 10.4103/0976-237X.137931
- [9] Irani, D.N., *Cerebrospinal Fluid in Clinical Practice*. Philadelphia, PA: Saunders/Elsevier; 2009. Chapter 10: Properties and Composition of Normal Cerebrospinal Fluid; pp. 69–89.
- [10] Kestin J, Sokolov M, Wakeham WA. Viscosity of liquid water in the range –8 °C to 150 °C. *Journal of Physical and Chemical Reference Data*. 1978;7(3): 941-948
- [11] Inman BA, Etienne W, Rubin R, et al. The impact of temperature and urinary constituents on urine viscosity and its relevance to bladder hyperthermia treatment. *International Journal of Hyperthermia*. 2013;29(3): 206-210. DOI: 10.3109/02656736.2013.775355
- [12] Haya L, Tavoularis S. Effects of bileaflet mechanical heart valve orientation on fluid stresses and coronary flow. *Journal of Fluid Mechanics*. Cambridge University Press. 2016;806:129-164
- [13] Tworoger SS. Collection, processing, and storage of biological samples in epidemiologic studies: Sex hormones, carotenoids, inflammatory markers, and proteomics as examples. *Cancer Epidemiology Biomarkers & Prevention*. 2006;15(9):1578-1581. DOI: 10.1158/1055-9965.epi-06-0629
- [14] Amadeu JK et al. Effect of storage time and temperature on salivary Total antioxidant capacity, Total oxidant status, and oxidant stress index. *Acta Stomatologica Croatica*. 2019;53(2):119-124. DOI: 10.15644/asc53/2/3

[15] Robles TF et al. Saliva sampling method affects performance of a salivary α -amylase biosensor. *American Journal of Human Biology*. 2013;25(6): 719-724. DOI: 10.1002/ajhb.22438

[16] Instructions for the use of The Cannon-Fenske Routine ... [Internet]. [cited 2020Jul25]. Available from: <https://www.cannoninstrument.com/Image/GetDocument/437?language=en>

[17] Johannsen B, Müller L, Baumgartner D, Karkossa L, Früh S, Bostanci N, et al. Automated Pre-Analytic Processing of Whole Saliva Using Magnet-Beating for Point-of-Care Protein Biomarker Analysis. *Micromachines* [Internet]. MDPI AG; 2019 Nov 30;10(12):833. Available from: <http://dx.doi.org/10.3390/mi10120833>

[18] Bansil R et al. Mucin biophysics. *Annual Review of Physiology*. 1995;57: 635-657

[19] Picout DR, Ross-Murphy SB. Rheology of biopolymer solutions and gels. *The Scientific World*. 2004;3: 105-121

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