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Rheology of stimulated whole saliva in a typical pre-orthodontic sample population*

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The dynamic viscosity (μ) of stimulated whole saliva in a typical pre-orthodontic sample population was characterized as a function of temperature (T). Samples were collected from 30 adolescents or young adults, after screening for factors that are known to have an effect on salivary viscosity. Using a cone and plate viscometer, 1.5 ml of stimulated whole saliva was evaluated at a constant shear rate of 450 s^{-1} from $T = 20\,^{\circ}C$ to $T = 40\,^{\circ}C$. Data from the μ–T plots showed a negative dependence of the form, $\mu = a - bT$, over a range of μ from 1.08 to 2.45 centipoise (cps) at 34 °C. Most of the samples fell into a narrow envelope, where the mean μ of the saliva samples ranged from 2.42 ± 0.61 cps at 20 °C to 1.57 ± 0.32 cps at 37 °C. With regard to sample stability, viscosity–time plots indicated that a small but predictable decrease in μ occurred during the 3 h period. The μ–T plots generated from fresh and frozen saliva samples demonstrated an appreciable change in μ as a result of refrigeration. With regard to sample reproducibility, viscometric data obtained from a typical pre-orthodontic patient over a 1-week period fluctuated within a fairly broad envelope of values.

1. Introduction

Human saliva is a complex and vital body fluid that is critical to good dental health. In addition to moistening the mucosa, aiding the digestive process, providing ions for remineralization, and chemically buffering the oral cavity, saliva provides lubrication of oral tissues [1–12]. The lubricating capacity of saliva has intuitively been correlated with the viscosity of the secretion by previous investigators [7, 12–15]. These lubricating properties are provided mainly by high molecular weight O- and N-linked glycoproteins that order water molecules and increase the viscosity of saliva beyond that of water [2, 5, 8–11, 15, 16].

In the orthodontic literature controversy surrounds what effect saliva may have on the components of a tooth moving system [17–32]. Further controversy has resulted from experiments involving artificial salivas and the friction generated by specific archwire/bracket combinations [20–22, 33].

Assuming that a correlation does exist between dynamic viscosity and oral lubrication, a specific objective was to measure the salivary viscosity of a typical pre-orthodontic population from samples obtained under well-delineated conditions. These samples are stimulated whole saliva—presumably a mixture of gland secretions, food, bacteria, sloughed tissues, white cells, etc.—and much the same as the saliva that an orthodontic appliance would be exposed to in a patient's oral cavity. In this investigation the effects of time, temperature and storage on viscosity are determined. Ultimately, we establish whether the inter- and intra-patient viscosities can provide a baseline so that the frictional coefficients of orthodontic archwire/bracket couples can be studied as a function of representative whole salivas.

2. Materials and methods

2.1. Sample procurement

Thirty-five individuals were screened via a written survey to ensure that certain criteria were met. These were that each person: (1) was between the ages of 10 and 40; (2) had no chronic illness; (3) took no medication on a regular basis; (4) had not experienced a cold or flu within the 2-week period prior to testing; (5) considered themselves to be in good health; and (6) had not eaten within 1 h prior to testing.

As an outcome of the survey, 30 individuals were asked to rinse with deionized water, chew on a 2″ × 2″ piece of Parafilm (American National Can, Greenwich, CT), and expectorate all available saliva into a cup for 10 min [34]. All samples were collected between the hours of 2:00 pm and 3:00 pm as earlier studies had indicated some diurnal variation in saliva production and presumably composition [4, 35, 36]. Both the collection time and the volume produced were recorded for all individuals so that flow rates could be calculated.

2.2. Viscometry

For each participant, a 1.5 ml aliquot of stimulated whole saliva was transferred into the sample cup of

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a Brookfield Digital Cone and Plate Viscometer Model LVTDV-II CP (Brookfield Engineering Laboratories, Inc., Stoughton, MA) that was interfaced with an IBM PC-30 (IBM, Boca Raton, FL) and a modified Lauda K4/R Electronic Water Circulator (Brinkman Instruments, Westbury, NY). With this apparatus (Fig. 1) the dynamic viscosity of each saliva sample was measured and recorded over the temperature range from 20 to 40 °C in 1 °C increments using a Model CP-40, 0.8 degree cone at a constant shear rate of 450 s^{-1}. This temperature range was chosen because orthodontic sliding mechanics are typically measured at 20, 34, or 37 °C [25, 27–29, 31, 37–39], while the shear rate was chosen because the viscosities are invariant for shear rates in excess of 100 s^{-1} [2, 5]. The viscometer was calibrated before each test series with Cannon Certified Viscosity Standard S3 (Cannon Instrument Co, State College, PA). the sample was maintained at each temperature step for 2 min before each viscometric reading was taken. The time required for an entire series of temperature steps was approximately 3 h. The viscometry apparatus and its operation are detailed in the Appendix.

2.3. Sample stability

To ensure that the measured changes in the viscometric data were caused by temperature and not time, three samples from one individual were maintained at 20, 30, and 40 °C by the water circulator. During the ensuing 3 h time period, viscometric readings were taken every 5 min utilizing the Brookfield DV Gather Software (Brookfield Engineering Laboratories, Inc., Stoughton, MA). Viscosity versus time plots were generated for each sample.

To determine the effect that storage via freezing has on these saliva samples, five samples were tested both before and after storage at − 20 °C. Viscosity versus temperature data were acquired after storage times that varied from 1 to 7 days.

2.4. Sample reproducibility

To ascertain the likely variability of salivary viscosity from a given individual, i.e. the intra-patient variation, one patient was provided a saliva sample on five consecutive days so that any differences in viscometric data could be measured.

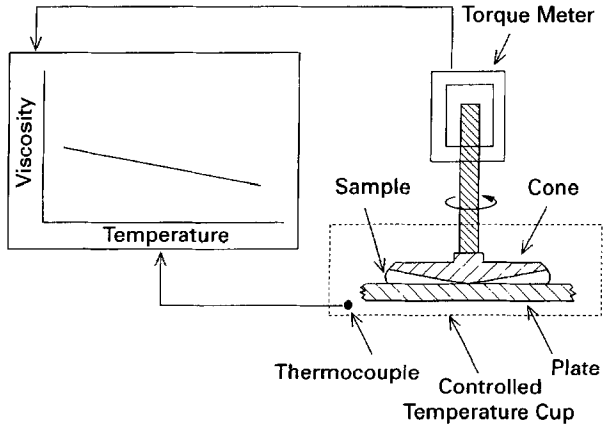

Figure 1 Schematic illustration of viscometric apparatus.

TABLE I Sample characteristics used in this rheological study of pre-orthodontic patients

Administered survey	35
Saliva sampled	30
Male	14
Female	16
Mean age (years ± s.d.)	23.6 ± 9.6
Age range (years)	11–39

2.5. Data analysis

The mean viscosities and their standard deviations at 20, 34, and 37 °C were calculated for all saliva samples from data shown in the viscosity versus temperature plots. Demographic information that was available from the written surveys—such as age, gender, dietary habits, and oral care routine—was used to create subgroups so that inferential statistics could be applied to the data as required (Table I).

3. Results

Under stimulated conditions, a mean salivary flow rate of 1.88 ± 0.01 ml/min was obtained for females, and 2.08 ± 0.01 ml/min was recorded for the males in the group ($p < 0.05$). The overall mean salivary flow rate was 1.98 ± 0.01 ml/min.

Data from the viscosity (μ) versus temperature (T) plots (Fig. 2) indicated that the viscosity characteristics of saliva show a negative dependence of the form, $\mu = a - bT$, over a rather narrow range from 1.08 to 2.45 cps at 34 °C (to convert centipoise into SI units: 1 cps = 1 mPa s). The viscosity values of the sample population converge from a broad band that averages 2.1 cps at 20 °C down to 1.2 cps at 40 °C. Although most of the data fell into a rather tight envelope, there was some indication that two μ–T profiles, A and B, were more prevalent (enclosed by brackets). No demographic information could account for this finding.

The mean viscosity of the saliva samples ranged from 2.42 ± 0.61 cps at 20 °C to 1.57 ± 0.32 cps at 37 °C (Table II). No significant difference was seen between the mean viscosities according to gender or age at any temperature.

With regard to sample stability, viscosity versus time plots indicated that a predictable but very small decrease in viscosity occurred during the 3 h testing period (Fig. 3). A plot of the change in viscosity versus temperature generated for the five fresh and frozen saliva samples indicated that, for all but one of the samples tested, a systematic drop of viscosity occurred that was attributed to the freezing process (Fig. 4a). A representative viscosity versus temperature plot generated from one set of fresh and frozen saliva samples demonstrated a 0.25 cps drop in viscosity across the entire temperature range (Fig. 4b). After

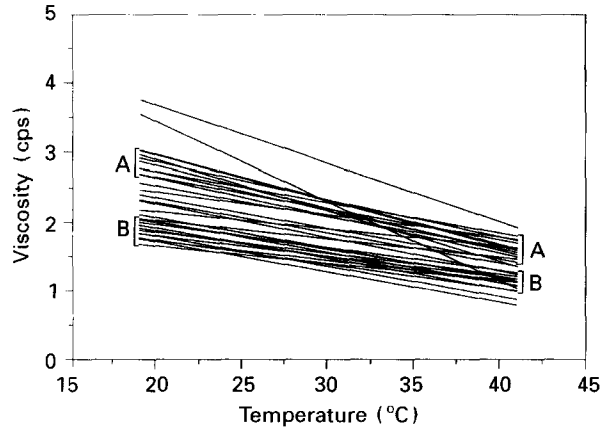

Figure 2 Viscosity–temperature plots of 30 saliva samples. Two sub-samples, A and B, were more prevalent.

TABLE II Influence of temperature on the viscosity of saliva of pre-orthodontic patients

Sample number	Viscosity (cps)		
	$T = 20\,^{\circ}C$	$T = 34\,^{\circ}C$	$T = 37\,^{\circ}C$
1	2.34	1.56	1.43
2	2.00	1.50	1.42
3	3.10	2.09	1.88
4	2.73	1.96	1.79
5	2.86	1.91	1.79
6	3.09	2.02	1.90
7	3.37	2.07	1.95
8	2.63	1.91	1.72
9	1.79	1.24	1.17
10	3.81	2.45	2.30
11	2.12	1.38	1.28
12	3.33	1.90	1.70
13	2.42	1.72	1.63
14	1.71	1.08	0.98
15	1.66	1.30	1.24
16	[a]	1.90	[a]
17	1.82	1.34	1.28
18	1.81	1.33	1.22
19	2.16	1.82	1.75
20	1.70	1.36	1.34
21	2.07	1.66	1.59
22	2.47	1.92	1.82
23	2.75	2.01	1.94
24	2.12	1.45	1.35
25	1.84	1.38	1.38
26	3.59	1.92	1.45
27	2.02	1.54	1.30
28	2.52	1.49	1.61
29	1.99	1.88	1.90
30	2.34	2.09	1.10
Mean	2.42	1.69	1.57
s.d.	0.61	0.33	0.32
Max. range	3.81	2.45	2.30
Min. range	1.66	1.08	0.98

[a]Denotes missing data.

freezing, no relationship was noted between the length of the storage time and the change in viscosity.

With regard to sample reproducibility, viscometric data obtained from an individual over the course of 1 week (Fig. 5) indicated that the daily variation fluctuated within a fairly broad envelope, although well within the envelope of the pre-orthodontic sample population examined (cf. Fig. 2).

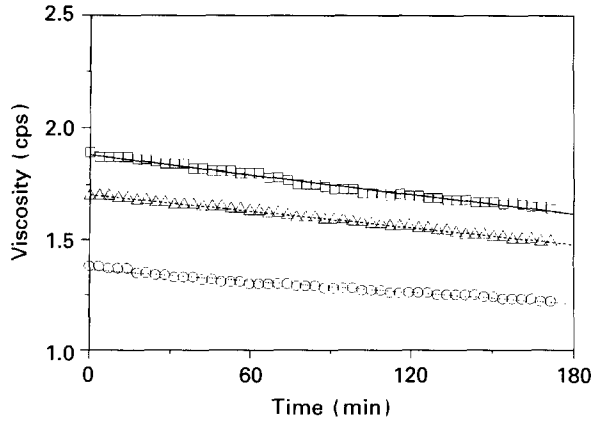

Figure 3 Viscosity–time plots of salivas evaluated for 3 h at three different constant temperatures: □, 20 °C; △, 30 °C; and ○, 40 °C.

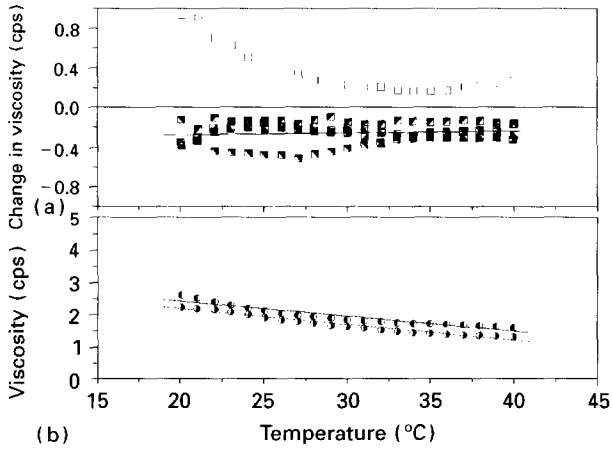

Figure 4(a) Change in viscosity–temperature plots of five saliva samples that were tested in the fresh and frozen states, after storage for 1 (◥), 2 (◪), 3 (□), 5 (◹), and 7 (◩) days. (b) Representative viscosity–temperature plots of a saliva sample that was tested in the fresh (◐) and frozen (◑) states, after storage for 5 days.

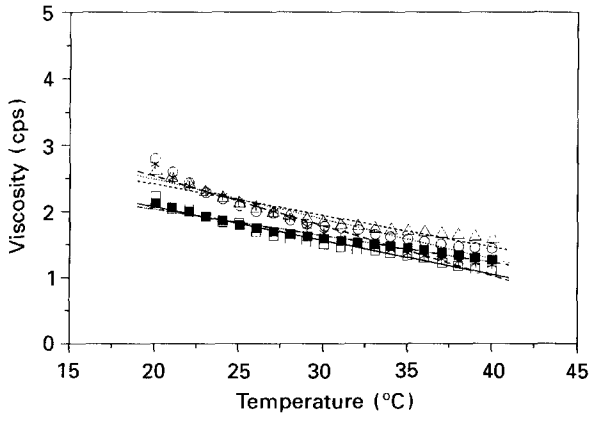

Figure 5 Viscosity–temperature plots of saliva samples collected on five consecutive days from one individual: □, day one; △, day two; ○, day three; *, day four; ■, day five.

4. Discussion

In general, prior measurements of salivary viscosities have varied considerably [1–3, 7, 15, 16, 35, 40]. Such differences have been associated with the source of the sample (whole versus glandular secretions), the manner of sample collecting and handling, the method and

time of testing, and individual variation in the chemical composition of the secretion [1–4, 15, 41, 42]. For example, stimulated saliva has a lower viscosity than unstimulated saliva samples [2]. By collecting a large number of samples under well-defined conditions, a good estimation of normal human salivary viscosity has been obtained. Our data supports other work, which has shown that males have higher salivary flow rates than females: (1) when mean values are considered [16]; and (2) in three out of four situations, when the lowest and highest tenth percentiles of salivas from stimulated parotid or submandibular glands—considered [43].

To verify specific aspects of the saliva evaluation technique, three experiments were done at the start of sample collection. They indicated that: (a) a small but consistent drop in viscosity values occurs over the 3 h testing period as a function of time and is independent of the temperature (Fig. 3); (b) frozen saliva samples are inadequate representations of fresh saliva, when stored at $-20\,^{\circ}C$ for 1 to 7 days (Fig. 4); and (c) individual daily variability in salivary viscosity appears great enough that one cannot assume an individual's salivary viscosity will stay within a small envelope of values on subsequent days (Fig. 5).

When sliding mechanics is used, the orthodontist's goal is to move teeth with the maximum efficiency and reproducibility. Together these two factors ensure that frictional forces and inter-patient variability are minimized. Furthermore, sliding mechanics is not only dependent upon whether the archwire/bracket couple is in a "dry" or "wet" state but also what the specific circumstances of the "wet" state are. With this clinical perspective in mind, we sought to establish the baseline properties of whole saliva for a pre-orthodontic sample population.

The physico-chemical role that saliva plays in frictional forces, which are inherent in orthodontic appliance systems, is not adequately understood. One surface rubbing against another, such as an archwire within a bracket, results in friction and causes wear. Lubrication consists of introducing an intermediate or boundary layer (the so-called third body) between the two surfaces to prevent contact. In order to determine the appropriate rheological properties of stimulated whole salivas—and in the future, of novel artificial salivas or additives that could enhance lubricity within the mouths of orthodontic patients—all contacting surfaces must first be modelled to represent the typical clinical situations in which the extents of motion between the various materials and the resulting tribological (i.e. friction and wear) characteristics are determined. First, however, the behaviour of saliva, as a lubricant in sliding mechanics, requires a thorough investigation of the normal patient population that we have described here. Within that context, specific questions must be answered. For example, how does the presence of a more viscous saliva affect the frictional forces present in a typical tooth moving system? Experiments designed to ascertain these relationships are now possible and are considered in a succeeding article.

Appendix

A cone and plate viscometer rotates a sensing element in a fluid and measures the torque necessary to overcome the viscous resistance to the induced movement. The sensing element, called a spindle, is driven by a spring. The magnitude of this resistance to twisting, which is detected by a rotational transducer, is proportional to the viscosity of the fluid. The viscometer is able to measure over a number of ranges since, for a given spring deflection, the actual viscosity is inversely proportional to the spindle speed. Shear stress is related to the spindle's size and shape.

In general,

$$\text{dynamic viscosity (cps or mPa s)} = \text{shear stress} \times 100/\text{shear rate} \quad \text{(A1)}$$

in which,

$$\text{shear stress (dyne/cm}^2) = \frac{\text{full-scale torque constant} \times \%\,\text{full-scale torque}}{2/3 \times \pi \times (\text{cone radius})^3} \quad \text{(A2)}$$

and

$$\text{shear rate s}^{-1} = \frac{\text{cone speed}}{\sin\,(\text{cone angle})} \quad \text{(A3)}$$

By substituting μ = dynamic viscosity, K = full-scale torque constant (673.7 dyne cm), T = per cent of full-scale torque, r = cone radius, ω = cone speed, and θ = cone angle into Equations A2 and A3, Equation A1 reduces to

$$\mu = \frac{150\,KT \times \sin\theta}{\pi^3 r^3 \omega} = \frac{3.218 \times 10^4\,T(\sin\theta)}{r^3\omega} \quad \text{(A4)}$$

By substituting the cone parameters ($r = 2.4$ cm, $\theta = 0.8°$, and $\omega = 2\pi$ rad/s) used in the present study, Equation A4 further simplifies to

$$\mu = 5.176\,T \quad \text{(A5)}$$

Hence, by measuring the per cent of full-scale torque as a function of temperature, the dynamic viscosity (which we simply label as "viscosity" in units of centipoise or "cps") is obtained as a function of temperature.

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