ORIGINAL

Modification of a simple device for measuring pH : demonstration of stable pH values and diurnal variation in human mixed saliva

Tadasi Sato

*Department of Oral Physiology, School of Dentistry, Iwate Medical
University, Morioka 020, Japan*

(Chairman : Prof. Takashi A. Suzuki)

[Accepted for publication : August 24, 1990]

Key words : Mixed saliva / pH / diurnal rhythm / breathing / basal pH

Abstract : After investigating the standard error of a plane glass electrode pH meter for a thin layer sample, the pH of human resting saliva collected between the tongue apex and the hard palate with a piece of neutral paper was studied in the following ways.

1. When a subject performed maximum forced nose breathing for 15 sec, the pH of the mixed saliva was little changed. In contrast it rose by about 0.2 points after the maximum forced mouth breathing for 15 sec. Thus, the following measurement was performed under conditions avoiding the error caused by mouth breathing and vigorous conversation.

2. The average pH value of the mixed saliva collected in the morning was significantly lower than that of samples (n=5) collected during the daytime.

3. The mean pH value of the mixed saliva from 5 diurnal cycles collected from a single subject indicated a reproducible diurnal change which appeared to consist of 1) a short-lasting variation (about ± 0.5 points) during 0.5-1.5 h after food-intake, and 2) a long-lasting variation (about -1 point) alternating from daytime to night time.

4. A significant finding was an abrupt increase in the pH level observed when changing from the covered mode to the uncovered mode. This increase appeared due to the $[\text{HCO}_3^-]$ change in the saliva and its peak came at approximately 4 min after changing the mode of measurement.

Introduction

It has been documented by many investigators that the pH of human mixed saliva changes with variations in 1) the concentration of carbon dioxide in the alveolar air and atrial blood¹⁻³⁾, 2) buffering activity⁴⁾, 3) quality of the food⁵⁾, and 4) secretion speed⁶⁻⁷⁾. In the clinical field, the relationship between the pH of human saliva and tartar formation⁸⁾ or sugar consumption and dental caries⁹⁻¹⁰⁾ has been investigated in detail. By using conventional measuring methods, however, it is difficult to design an experiment so that

the human subject can avoid discomfort when the samples are collected during the day and night over a period of several days. Thus, such research has not been done extensively. Recently, a card type of pH meter which may overcome such difficulties has become commercially available. Therefore, in this study, the applicability of this new pH meter to the study of human saliva was investigated. A part of the present results have been reported in abstract form¹¹⁻¹²⁾.

Materials and Methods

The pH of each sample was measured with

a digital pH meter (Horiba C-1; Horiba Ltd., Tokyo) utilizing the newly designed plane glass pH electrode to measure the thin layer sample. With the sensor being located at the bottom of a shallow measuring pool of about 1 mm depth, the test solution was sampled with a piece of neutral sampling paper (Sampling Sheet YO 11, Horiba Ltd.; $15 \times 10 \text{ mm}^2$) which was placed on the pH electrode. The pH meter was calibrated with a set of standard solutions of pH 6.86 and 4.01.

The sample of the mixed saliva was collected intraorally by using the sampling paper which had been placed on the dorsal surface of the tongue apex. This paper was made wet with the mixed saliva which was located between the tongue apex and the hard palate. In some experiments, the mixed saliva was expectorated through a polyethylene tube (OD 2 mm; ID 1.5 mm) for 5-10 min and stored in a bottle underneath liquid paraffin at room temperature before being used. The saliva was collected at rest from 5 healthy persons ranging between 15 and 45 years of age, who had brushed their teeth at least once before sleeping.

Stability of pH Measurement

In order to investigate the stability of the pH measurement, the time-dependent change in the displayed value of the pH was recorded 5 times for a standard solution of pH 6.86 and a 0.9 % NaCl solution as shown in Fig. 1 A. For the standard solution, the difference between the readout value and the final value after 5 min of recording became less than 1 % 10 sec after the start of the recording. The mean standard error of the present pH measurement was 0.3 % of the final pH value after 5 min of recording (Fig. 1 A-a). In the case of the 0.9 % NaCl solution (not containing buffer) the pH value elevated rapidly 20 sec after the start of the recording and then tended to elevate gradually (Fig. 1 A-b). The mean standard error was 4.7 % of the final value in this case, while the mean standard error became 1/2.5 of the former error after the surface of the sample was covered with liquid paraffin (Fig. 1 A-c). As the magnitude of the standard error and the discrepancy in the mean pH

values between curves b and c of Figure 1 A was relatively large during the first 30 sec, a stable pH value with this device could not be obtained during the first 30 sec if the buffering activity of the sample was very weak.

Time-Dependent Change in pH of the Mixed Resting-Saliva

Aliquots of about $60 \mu\text{l}$ of the resting saliva stored in a bottle were used for the pH measurement. The pH of the mixed resting-saliva increased gradually after the start of recording, and the final value was about 0.6 points higher than the initial value (Fig. 1 B-a). The mean value of the standard error at each testing point was about 0.8 % of the final value, when 5 aliquots from the same sample were tested. This value was clearly smaller than that for the 0.9 % NaCl sample. However, it was hard to record a stable pH value of the saliva by the usual measuring method because of the continuous increase in the displayed value during the 5-min measurement; this was probably due to the volatilization of CO_2 from the thin layer sample. Therefore, the surface of the sample was covered with liquid paraffin (Fig. 1 B-b) or a piece of oil paper (Fig. 1 B-c) and with a standard error of 0.7 % a stable pH value was readily obtained. As the use of oil paper or a plastic plate is convenient for frequent measurements and the values obtained are comparable to those obtained for liquid paraffin, the following pH measurements in the covered mode were performed by using either oil paper or a plastic plate. The values obtained in the uncovered mode were recorded at about 30 sec after the start of the recording, since the recorded values were approximately the same at this time irrespective of the measuring mode as shown in Fig. 1 B.

Results

Effect of Forced Ventilation on the pH Value of the Mixed Saliva

The pH value of the saliva from one subject did not change significantly after maximum nose breathing for 15 sec (Fig. 2 A), while it elevated about 0.2 points for 2-3 min after maximum mouth breathing (Fig. 2 B). The

Fig. 1 Temporal change in displayed value of the digital pH meter. The sample of curve A-a was a standard solution of pH 6.86 and that of A-b was a 0.9 % NaCl solution. These were tested in the usual way where the surface of the sample was uncovered, whereas a sample from the same NaCl solution was tested in the case of curve A-c when the surface of the solution was covered with liquid paraffin.

In panel B, aliquots taken from the same sample of resting-saliva were tested, but by different measurement methods in that the surface of the sample was uncovered (B-a), covered with liquid paraffin (B-b), and covered with a piece of oil paper (B-c) instead of liquid paraffin. Abscissae: minutes; coordinates: pH. Vertical bars on each curve indicate standard error of the meter for each measurement condition. The vertical bars were drawn only when the range exceeded one and a half times the radius of each measured point.

mean standard errors of pH measured in the covered mode for both breathing procedures (Figs. 2 A-b and 2 B-b) were 20-50 % smaller than those in the uncovered mode (Figs. 2 A-a and 2 B-a).

pH Value of Mixed Resting-Saliva Just Before Rising, Also Before and After Meals

Fig. 2 Panels A & B indicate temporal change in pH of the mixed saliva when the surface of the sample was uncovered (A-a, B-a) or covered with a piece of oil paper (A-b, B-b) following maximal nose breathing (panel A) or maximal mouth breathing (panel B) for 15 sec at a time indicated with an arrow. Vertical bars: standard deviation of 5 samples collected from the same subject.

Without the pH elevation caused by mouth breathing or vigorous conversation, the samples of resting-saliva which were collected from 5 subjects in the morning just before rising from bed (basal pH value) were analyzed for 5 days. The mean pH value fluctuated little in comparison with the range of the standard deviation (Fig. 3 A). The standard deviation of the daily data for each person was 27 % smaller than that of all subjects (Table 1).

The pH's of the saliva measured before and after meals were all significantly higher than those of the basal value ($p < 0.01$, Student's t-test), but the deviations were smaller than that of the basal value (Fig. 3 B). In addition, the pH values of the resting-saliva tended to decrease after meals, though they were not found statistically significant.

Circadian Rhythm in pH of the Resting-Saliva

In order to investigate whether a circadian change could be detected, the pH value of the resting-saliva of one subject was recorded

every 1 h in the daytime, and every 4-5 h in the night time. It was found that the pH value decreased significantly in the night time (Fig. 4) as compared to the daytime value. Thus, the measurement was continued for 4 more days. The pH value of the resting-saliva increased by about 0.5-1.0 points after rising from bed, stayed at these levels during the daytime, and then went down about 1.0

Fig. 3 Daily change in basal pH of the mixed saliva (panel A) sampled from 5 subjects just before getting out of bed, and comparison between the basal (B) and daily pH of the mixed saliva (panel B) sampled from 5 subjects just before a meal (BM), and 30 min and 60 min after a meal (AM). Vertical bars: standard deviation.

point in the night time (Fig. 5 A). This reproducible rhythmicity was evident in the graph of mean values (Fig. 5 B) after minimizing the meal-dependent transient changes of about ± 0.5 points by means of an averaging summation.

pH Variation of the Resting-Saliva during the Recording in the Uncovered Mode

As the pH of the saliva rose with time as shown Fig. 1 B-a, this elevating time course was investigated by increasing the sampling frequency and switching the measuring mode from the covered to the uncovered. A steady pH value of the mixed saliva was recorded from 30 sec to 5 min in the covered mode utilizing a piece of plastic plate, and then an abrupt increment of pH appeared after switching the measuring method from the covered to the uncovered mode (Fig. 6 A). This increment in pH was clearly different from the initial instability of the pH meter or the temperature decrease of the sample due to transfer from the intraoral site to the surface of the pH electrode. The pH elevation terminated at about 4-5 min after removing the plastic plate from the surface of the sample. When this measurement was performed 5 times with 15-min intervals, a marked deviation was observed as indicated by the vertical bars of Fig. 6 A. In order to examine whether the deviation was mainly due to the error of this measurement method or to the physiological fluctuation in salivary secretion, the same measurement was carried out on the saliva discharged through a polyethylene tube and stored in a bottle underneath

Table 1 Basal value in pH of mixed saliva.

subject	Date					mean	SD
	1	2	3	4	5		
1	7.35	7.17	7.18	7.31	7.98	7.40	0.30
2	6.76	6.40	6.95	6.53	6.52	6.63	0.20
3	5.85	6.78	6.12	6.26	6.86	6.37	0.39
4	6.57	6.64	7.18	6.21	7.18	6.76	0.38
5	6.97	6.16	7.11	6.72	6.72	6.74	0.32
mean	6.70	6.63	6.91	6.61	7.05	6.78	0.32 ^a
SD	0.50	0.34	0.40	0.40	0.51	0.43 ^b	

a : SD for each subject

b : SD of all subjects

Fig. 4 Diurnal change in pH of mixed saliva. Samples were collected from one subject every hour in the daytime while every 4-5 h in the night time. Arrows indicate the time of meal, and the horizontal bar indicates the period of sleep.

Fig. 5 Panel A: same as Fig. 4 but represents data collected for 5 cumulative days from one subject. Panel B shows the diurnal change in pH of the mixed saliva with the mean value and the standard deviation. The data came from panel A.

Fig. 6 Temporal change in pH of the mixed saliva by switching the method of measurement from the covered mode (filled bar) to the uncovered mode (open bar) at 5 min. 60 μ l samples for panel A were collected 5 times at 15-min intervals from one subject. A single 2-3 ml sample for panel B was collected from one subject and stored in a bottle underneath floating liquid paraffin at room temperature for approximately 75 min during which time five 60 μ l samples were drawn and measured as in panel A.

liquid paraffin at room temperature (Fig. 6 B). The standard deviation of the latter sample was about half of those recorded in Fig. 6 A. Thus, the standard deviation in Fig. 6 A seems to include both physiological fluctuations in the salivary secretion as well as errors due to this form of pH measurement.

Discussion

The properties of saliva have been well described from physicochemical and physiological¹³⁻¹⁴⁾, as well as clinical^{2,15-16)} viewpoints. However, the pH of saliva has not been adopted as a diagnostic index since it is difficult to collect enough volume of the resting saliva because of the low secretion speed at rest. Moreover, the pH value of the

saliva tends to be unstable, because the method used for its collection permits the unwanted effects of the volatile CO_2 escaping from the saliva. Thus, the possibility of overcoming the difficulty stated above was described in the present paper.

Measuring Mode of pH

The Horiba C-1 pH meter is designed to measure thin layer samples. Thus, stable pH values could be readily recorded under an equilibrium state at room temperature and at the Pco_2 of the room air. However, it was hard to record stable pH values of the mixed saliva since the displayed value elevated continuously during the 5-min measurement as shown in Fig. 1 B-a. Moreover, it was difficult to determine the main cause of the continuous elevation in pH of the saliva among such factors as initial instability of the pH meter, temperature decrease of the sample, volatilization of CO_2 gas from the sample, and weakness of the buffer in the sample. After several trials and errors, by switching from the covered to the uncovered measuring mode after termination of change in the displayed value due to the initial instability of the pH meter, it was determined that the pH elevation was probably due to the volatilization of the CO_2 in the saliva and not due to the other two factors (see Fig. 6). Therefore, an accurate pH value of the salivary sample can be obtained with this pH meter in the covered mode or just after termination of the initial instability period and/or temperature change in the first 30 sec in the uncovered mode. In addition, collecting a sample just after mouth breathing (Fig. 2 B) or vigorous conversation should be avoided.

Circadian Change in pH of the Mixed Saliva

The pH of saliva is dependent largely upon secretion speed⁶⁻⁷⁾, and the secretion speed is extremely low in the sleeping period¹⁹⁾. Thus, the fall of the pH during sleep in Fig. 5 A seems to be a result of a decrease in the flow rate of saliva, and the pH elevation after rising from bed is the result of an increase in the flow rate.

Circadian change in the pH of saliva has been reported by Ferguson and his coworkers for stimulated parotid saliva²⁰⁻²¹⁾ and resting

submandibular saliva²²⁾ as a less variable and less reproducible parameter than that of the oral temperature or of the salivary flow rate. Moreover, their results for the pH of resting submandibular saliva have not been well correlated with changes in salivary flow rate associated with alternation in the duration and phase of sleep and being awake²³⁾. However the present results seem to agree well with the change in the flow rate and to indicate reproducible cyclic changes as shown in Fig. 5 A. In addition, the daily change in the pH of the mixed saliva was confirmed after reducing the transient changes in pH by averaging summation (Fig. 5 B). One possible reason for the poor reproducibility for the salivary pH reported previously may be due to the sample volume and collecting time. Ferguson and Fort²²⁾ collected samples of 3 ml even during the sleeping period when the flow rate is at its lowest. In the present method, these values were 3 ml vs. 60 μl (50 times smaller) and 30 min vs. 15 sec (120 times faster). Therefore, the present method has the important advantage of being able to record the circadian change in pH of the saliva with respect to the minimum and necessary volume and its collecting time. Recording the circadian change for several days is also possible under the least invasive conditions.

pH of Mixed Saliva and Buffering Activity

In the clinical field of dental medicine, it has been reported that the pH level of the mixed saliva is secondarily correlated with the formation of tartar⁸⁾ and dental caries⁹⁾. Moreover, the inspection of salivary pH has been found to have diagnostic value in patients with diabetes mellitus²⁴⁾ and gastric diseases^{14, 24-25)}.

In contrast, the buffering activity of the saliva has been described as having an inverse relation between the pH of the saliva and the CO_2 concentration in the alveolar air¹⁾ or CO_2 binding capacity of the blood²⁻³⁾. Lillenthal⁴⁾ has also reported that the buffering activity of the saliva is mainly dependent on the $[\text{CO}_2]$ and $[\text{H}_3\text{PO}_4]$. Thus, it appears important to study further the pH elevation compared to the baseline in the covered mode

in relation to the peak pH value measured in the uncovered mode (Fig. 6), which might be related to the CO_2 loss from the sample or an decrease in the $[\text{HCO}_3^-]$ of the sample. Although further experiments are required to compare this value with the $[\text{HCO}_3^-]$ change in saliva measured by direct measurement, the present method seems to have the advantage that an increase in sampling frequency is possible under clinical conditions and it is possible to trace the physiological change in the $[\text{HCO}_3^-]$ of the saliva in addition to the measurement of the salivary pH.

Acknowledgment

The author expresses great appreciation to the Chairman of The Department of Oral Physiology, Professor Takashi A. Sukuki and Chairman of the Department of Oral Hygiene, Professor Tsuyoshi Katayama, for their valuable criticism and advice; thanks also to Dr. Norio Matsumoto, Dr. Paul Langman, and Ms. Virginia Lee Braymer for their critical reading of the manuscript, Mrs. Shizuko Urata for her secretarial work, and the author's family members for their cooperation in collecting samples of mixed saliva.

抄録: 薄層標本測定用の平面ガラス電極型 pH メータを使用し、中性紙片で採取した安静時の舌背口蓋間唾液の pH を調べ、以下の成績を得た。

1. 口腔を介して15秒間の最大努力呼吸を行った場合に唾液 pH は呼吸終了後2～3分間約0.2だけ上昇した。そこで口呼吸や会話による影響を避けながら以下の測定を行った。
2. 早朝起床前の混合唾液の pH は日中の食前あるいは食後60分までの間に測定した値より有意に低い値であった。
3. 1被験者の5日間の混合唾液 pH の平均値は、食後0.5～1.5時間における pH 振幅約±0.5の一過性の変動と覚醒時に高く睡眠時に低い pH 振幅が約1の長期的な変動との組み合わせであった。
4. 本 pH メータの測定様式を被覆から非被覆様式に切り替えることにより唾液の pH の他にその緩衝能の指標と成り得る値を数分間で測定出来ることが示唆された。

References

- 1) Starr, H. E.: Studies of human mixed saliva. II. Variations in the hydrogen ion concentration of human mixed saliva. *J. Biol. Chem.* **54**: 55-64, 1922.
- 2) Watanabe, I.: Über den engen Zusammenhang zwischen der Wasserstoffionenkonzentration des Speichels und der Alkalireserve des Blutes beim Menschen. *Tokyo J. Med. Sci.* **54**: 886-895, 1940 a. (in Japanese).
- 3) Watanabe, I.: Experimentelle untersuchungen über den engen Zusammenhang zwischen der Wasserstoffionenkonzentration des Speichels und der Alkalireserve des Blutes beim Kaninchen. *Tokyo J. Med. Sci.* **54**: 896-905, 1940 b. (in Japanese).
- 4) Lilienthal, B.: An analysis of the buffer systems in saliva. *J. Dent. Res.* **34**: 516-530, 1955.
- 5) Ericsson, Y.: Clinical investigations of the salivary buffering action. *Acta Odontol. Scand.* **17**: 131-165, 1959.
- 6) Iwasaki, H.: Role of carbonic anhydrase in the alkali excretion of salivary gland. II. Report. Carbonic anhydrase contents of salivary glands and excretion of alkali in saliva. *J. Physiol. Soc. Jpn.* **19**: 1239-1256, 1957. (in Japanese).
- 7) Dawes, C. and Jenkins, G. N.: The effects of different stimuli on the composition of saliva in man. *J. Physiol. (Lond.)* **170**: 86-100, 1964.
- 8) Naeslund, C.: Studies of tartar formation. *Acta Path. Microbiol. Scand.* **3**: 637-677, 1926.
- 9) Fosdick, L. S.: Oral Medicine: Some new concepts concerning the role of sugar in dental caries. *Oral Surg.* **5**: 615-624, 1952.
- 10) Rugg-Gunn, A. J.: Diet and dental caries. In: *Frontiers of Oral Physiology*, (ed. by Ferguson, D. B.), Vol. 3, pp. 53-65, Karger, Basel, 1981.
- 11) Sato, T., Somei, K., Kawarada, K., Ooe, M. and Suzuki, T. A.: An application of digital pH meter of card type for study of change in pH of saliva. *Jpn. J. Oral Biol.* **30**(Suppl.): 127, 1988. (in Japanese).
- 12) Sato, T.: Intraoral positional difference in

- the time-dependent pH curve and diurnal variation. *Jpn. J. Oral Biol.* 31(Suppl.) : 90, 1989. (in Japanese).
- 13) Yoshimura, H. : Mechanism of acid-base secretion of digestive glands. *J. Physiol. Soc. Jpn.* 16 : 105-114, 1954. (in Japanese).
 - 14) Ferguson, D. B. : Physiological, pathological and pharmacologic variations in salivary composition. In : *Frontiers of Oral Physiology*, (ed. by Ferguson, D. B.), Vol. 3, pp. 138-153, Karger, Basel, 1981.
 - 15) Umeda, E., Kokufu, T. and Nasu, T. : Statistical observation about acidity of gastric juice in patient of diabetic mellitus. *Jpn. J. Gastroenterol.* 38 : 743-748, 1939. (in Japanese).
 - 16) Reindel, W. : Über die Gesetzmässige Gegensätzlichkeit zwischen den Aciditätsverhältnissen von Mundspeichel und Magensaft. *Wien, klin. Wschr.* 19 : 390-392, 1940.
 - 17) Krasnow, F. : Biochemical studies of dental caries : diurnal variations in the phosphorus, calcium, hydrogen-ion concentration, and titratable alkalinity. *J. Dent. Res.* 12 : 530-532, 1932.
 - 18) Krasnow, F. : Biochemical analysis of saliva in relation to caries. *Dent. Cosmos* 78 : 301-310, 1936.
 - 19) Schneyer, L. H., Pigman, W., Hanahan, L. B. and Gilmore, R. W. : Rate of flow of human parotid, sublingual, and submaxillary secretions during sleep. *J. Dent. Res.* 35 : 109-114, 1956.
 - 20) Ferguson, D. B., Elliott, A. L. and Potts, A. J. : Variations in human parotid saliva over 24-hour periods. *J. Dent. Res.* 48 : 1132, 1969.
 - 21) Ferguson, D. B., Fort, A., Elliott, A. L. and Potts, A. J. : Circadian rhythms in human parotid saliva flow rate and composition. *Arch. Oral Biol.* 18 : 1155-1173, 1973.
 - 22) Ferguson, D. B. and Fort, A. : Circadian variations in human resting submandibular saliva flow rate and composition. *Arch. Oral Biol.* 19 : 47-55, 1974.
 - 23) Dawes, C. : Circadian rhythms in human salivary flow rate and composition. *J. Physiol. (Lond.)* 220 : 529-545, 1972.
 - 24) Masuda, S. : Concentration of hydrogen ion of saliva and acidity of the gastric juice. *J. Med. Soc. Toho* 32 : 634-638, 1943. (in Japanese).
 - 25) Mayama, S., Mizusawa, T. and Tsunokake, S. : A study of the relationship between the acidity of gastric juice and the pH of saliva. *J. Iwate Med. Assoc.* 4 : 163-165, 1953. (in Japanese).