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Continuous measurement of intra-oral pH and temperature: development, validation of an appliance and a pilot study

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SUMMARY To describe a novel approach for continuous measurement of intra-oral pH and temperature in individuals carrying out normal daily activities over 24 h. We designed, validated and constructed a custom-made appliance fitted with a pH probe and a thermocouple. Six subjects wore the appliance over a 24-h period for two non-consecutive days, while the intra-oral pH and temperature were measured continuously and recorded. Intra-oral pH and temperature were very similar across different recording days, the difference being not statistically significant ($P \geq 0{\cdot}14$). There was a noticeable difference in the pattern of variation of pH between day and night. During the day, the mean pH was 7·3 (±0·4) and dropped markedly only after consumption of acidic food and drinks. The intra-oral pH decreased slowly during sleep with an average pH of 6·6 (±0·4) being recorded. The difference between day and night was statistically significant ($P = 0{\cdot}002$). The mean intra-oral temperature was 33·9 °C (±0·9) during daytime and 35·9 °C (±0·5) during sleep ($P = 0{\cdot}013$) with minor fluctuations occurring over 24 h. The continuous and simultaneous intra-oral pH and temperature measurement system described in this report is reliable, easy to construct, able to measure variables over a sustained period and may serve as a future diagnostic tool in a number of applications.

KEYWORDS: intra-oral pH, salivary pH, intra-oral temperature, dental erosion, circadian rhythm

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Introduction

In one of the earliest studies of dental erosion, Black (1) estimated that the condition was in fact rare with a 0·1% prevalence and resulted from a hereditary predisposition. Nowadays, there is a large body of evidence indicating that dental erosion has become much more prevalent, especially in younger individuals (2–5), and appears to be lifestyle-driven (6, 7).

It is generally recognised that erosion is a multifactorial condition, with both patient-related (e.g. salivary flow rate and buffering capacity) and nutritional factors (e.g. carbonated drinks) leading to tooth dissolution (8). Based on the premise that the development and progression of dental erosion depends on the intensity of acid exposure, a large number of studies have been undertaken. Classically, such studies have focused on the short-term measurement of pH values on selected tooth surfaces (9). Explaining the dynamics of dental erosion, however, extends beyond documenting differences in site-specific pH readings in persons with and without erosion. What is needed is an understanding of both the intensity, the duration of the acid exposure, and the buffering capacity of saliva that can only be understood by long-term monitoring in real-life settings.

Another factor that has not been investigated in dental erosion studies is intra-oral temperature. When

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intra-oral temperature is decreased, for instance by mouth breathing, saliva dries out, leading to a small amount of saliva in the mouth to buffer the existing acids. Despite its close relationship, previous research interests have primarily focused on measuring the temperature of ingested drinks and their impact on tooth surfaces and restorations rather than the temperature dynamics inside the oral cavity (9, 10).

Great efforts have been made in developing methods for measuring intra-oral pH and temperature over sustained periods. In early research, salivary pH measurements were performed *in vitro*. More recently, intra-oral pH has been assessed by means of biotelemetry using indwelling microelectrodes that were attached either to oral prostheses or directly to the dentition (11, 12). Gudmundsson *et al.* (11) simultaneously measured the intra-oral pH and oesophageal pH for 24 h. The small number of subjects studied and the probe site represented important limitations of the study. Indeed, pH was measured at the buccal surface of the first mandibular molar, which is a position where dental erosion rarely occurs, especially in patients with gastroesophageal reflux disease (GERD) (13). Davidson *et al.* (12) also measured the intra-oral pH of subjects during eating using a more efficient design, but testing was only carried out for several minutes. These issues were overcome by a wireless indwelling electrode introduced by Watanabe *et al.* (14) and Ro *et al.* (15). They successfully measured the intra-oral pH for 19 and 24 h, respectively; however, the measurements were limited to one subject only and there are no known follow-up clinical trials that have been carried out using their wireless telemetric devices to measure intra-oral pH.

For temperature measurement, thermocouple and thermistors have been widely used for intra-oral use. Previous studies report the measurement of oral temperature from a few minutes to 24 h (16, 17). There is only one study that has continuously measured intra-oral temperature over an extended duration in a large number of subjects (17). In addition, to the best of the author's knowledge, no one has reported the continuous and simultaneous measurement of intra-oral pH and temperatures for extended hours.

The ability to continuously measure the intra-oral pH and temperature for extended hours is clearly vital, because it could give insights into dental erosion, as well as many other disorders related to the intra-oral pH and temperature. Hence, an appliance overcoming the limitations previously reported will allow researchers to relate the intra-oral pH and temperature to other less studied factors such as circadian rhythms.

To do so, we firstly set an objective to develop and validate a novel device that could continuously and simultaneously measure pH and temperature intra-orally. In this article, we present the outcome of this development and the preliminary results from our investigation using the appliance in healthy individuals for a continuous period of 24 h.

Methods

Validation of the accuracy and temperature dependence of pH probe

An *in vitro* study was conducted to test the accuracy of a commercial pH equipment* and its temperature dependence using a professional pH meter with a glass electrode (PL700-PV[†]) as gold standard.

For the accuracy test, six solutions of various pH were prepared: (i) lemonade, (Charlies[‡]); (ii) orange juice[§]; (iii) Coca-Cola[¶]; (iv) antibacterial liquid hand soap, (**); (v) bottled water (Spring Water[††]); and (vi) liquid sodium hypochlorite (Bleach regular, Budget[‡‡]). After calibration of both pH probes according to manufacturer's instructions, the pH of 20 mL of each solution was recorded at the end of every minute, for 3 min. The probes were rinsed with distilled water between each solution to avoid contamination. The probes were calibrated and the procedures were repeated. The temperature of all drinks were measured and kept the same temperature throughout the experiment, which was 20·6 °C.

Accuracy of pH measurement can be influenced by changes of temperature. Therefore, an *in vitro* study was conducted to measure the temperature dependence of the Restech pH probe in comparison with the reference standard glass electrode at three differ-

*ResTech Corp, San Diego, CA, USA.
†GOnDO Electronic Co., Ltd, Taipei, Taiwan.
‡The better drinks Co Pty Ltd, Renmark, SA, Australia.
§Keri Juice Company, Auckland, New Zealand.
¶The Coca-Cola Company, Auckland, New Zealand.
**Dettol, Auckland, New Zealand.
††Pump Putaruru Water Ltd, Auckland, New Zealand.
‡‡Foodstuffs, Auckland, New Zealand.

ent temperatures. Three water baths (Julabo® water bath[§§]) were set to temperatures of 27, 37 and 47 °C, respectively. A cylindrical glass container, with 100 mL of phosphate-buffered saline solution prepared from a phosphate-buffered saline tablet (pH 7·4 at 25 °C[¶¶]), was placed into each water bath. A laboratory thermometer (Brannan thermometers***) was also placed in each cylindrical glass container to ensure that the temperature of the phosphate-buffered saline matched the temperature of the water bath. Prior to the experiment, the pH electrode was calibrated in a pH 4 and pH 7 buffer solutions as per manufacturer's instructions. Following calibration, the Restech pH probe and glass electrode were first immersed into the phosphate buffer saline solution in the 27 °C water bath for 10 min and checked that they produced the correct pH readings of 7·4. This was repeated with the 37 and 47 °C baths in a staircase function. This procedure was repeated three times. Once the temperature dependence of the pH probe was measured, the readings could then be compensated for temperature and be compared to the pH readings from the gold standard electrode.

Intra-oral appliance

Our appliance was designed to provide a thin flexible apparatus for the continuous and simultaneous measurement of both intra-oral pH and temperature at various locations in the mouth and was constructed with soft bleaching tray materials (BIO-BLEACH 1·0 × 125 mm[†††]). A maxillary alginate impression (Aroma Fine Plus[‡‡‡]) was taken in light-cured acrylic special trays (Plaque Photo[§§§]) and cast in vacuum mixed Type III dental stone (Hydrocal 105[¶¶¶]). A 1·0 × 125 mm layer of thermoplastic soft bleaching tray material (BIO-BLEACH[†††]) was then applied on the working cast with a pressure-moulding machine (Twin Star V[†††]). The applied sheet was trimmed leaving a 2–3 mm margin along the gingival margins of the teeth and along the posterior borders, covering one quadrant of dentition and the hard palate.

A pH probe* and a thermocouple (K-Type****) were secured on the first layer of BIO-BLEACH[†††] using a dental utility wax (Utility Wax Strips[††††]). For this study, the probe and thermocouple were placed in a single position, palatal side of an upper central incisor. The tube and wires from the measuring components (total length = approx. 75 cm) were positioned to wrap around the distal of the last molar and out of the mouth, to minimise occlusal interferences in centric and lateral excursive movements (Figs 1 and 2). The tips of the probe and thermocouple were then covered with a small piece of a ceramic ring liner (New Casting Liner – Dry Type[‡‡‡‡]) for protection from the heat generated by the pressure-moulding machine. A second layer of BIO-BLEACH[†††] was applied, bonding to the first layer and trimmed according to the outline of the previously applied layer. The edges were then smoothed and rounded with a heated wax knife to improve the comfort when being worn intra-orally. The area covering the probe and thermocouple was exposed by carefully cutting away the second layer of BIO-BLEACH[†††] and removing the ceramic ring liner. The pH probe and thermocouple were then individually connected to their own transmitter and calibrated according to manufacturers' instructions (Fig. 2).

Testing protocol

After obtaining ethical approval (University of Otago Human Ethics Committee H14/012), 10 healthy female volunteers from the pool of postgraduate students at the Faculty of Dentistry, University of Otago, agreed to participate in the study. The subjects were given a questionnaire to evaluate their health status. Those who gave positive answer to the followings conditions were excluded from the study: history of dental erosion, xerostomia, eating disorders, respiratory disorders, sleep disorders, allergy, intake of medication, mouth breathing, smoking, wearing of orthodontic appliances and restorations on upper anterior teeth. Six participants (mean age

[§§]JULABO GmbH, Seelbach, Germany.
[¶¶]Sigma-Aldrich®, St. Louis, MO, USA.
***Cleator Moor, Cumbria, UK.
[†††]Scheu Dental GmbH, Iserlohn, Germany.
[‡‡‡]GC, Tokyo, Japan.
[§§§]W+P Dental, Barmstedt, Germany.
[¶¶¶]USA Gypsum Co., Chicago, IL, USA.

****Lascar Electronics Inc., Erie, PA, USA.
[††††]Heraeus Kulzer, Armonk, NY, USA.
[‡‡‡‡]GC, Tokyo, Japan.

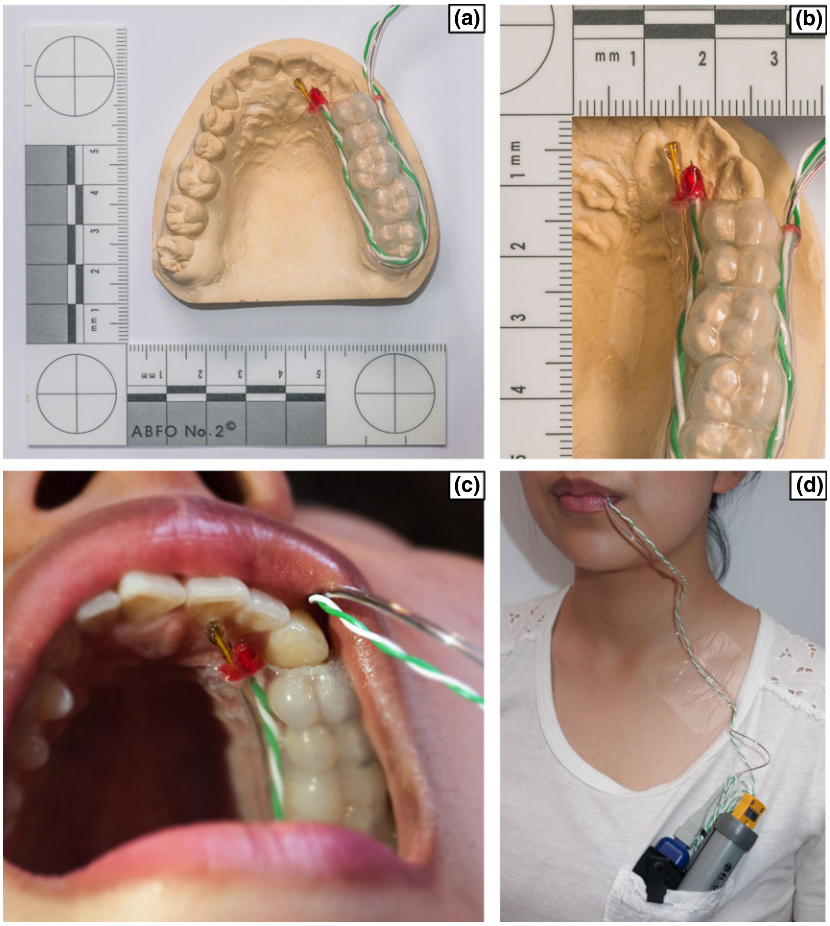

Fig. 1. Photograph showing (a, b) the design and positioning of pH probe and thermocouple, (c) the device in the mouth and (d) data transmitter in position.

Fig. 2. Photograph showing the design of our intra-oral pH and temperature measuring device (left side) connected by wires to the data recording devices (middle) and data display set (right side).

28·5 ± 3·9 years old) fulfilled the inclusion criteria and were selected. The subjects wore the appliance for two sessions of 24 h, which were non-consecutive and separated by 1 week apart. Prior to each experiment, the unstimulated saliva flow rate was measured using the spitting technique (18), whereby participants spit into a pre-weighed container every 30 s for a 5-min period. The container was weighted immediately after saliva collection, and the salivary flow rate was determined based on 1 g = 1 mL saliva (18). Once the experiment started, each participant wore the appliance continuously except at meal times and in the shower (Fig. 1). This was performed to avoid food residues getting into the probe or water getting into the data transmitter. The appliance was kept in distilled water at room temperature when not being worn, so that the pH probe would not dry out. All participants were asked to keep a detailed log of daily activities. Once completed, the pH results were transferred to a computer and analysed using View Lite software (View Lite*), while the temperature data were loaded onto a computer from the EasyLog USB**** memory device.

Data analysis

Data were collected and analysed using Microsoft Excel§§§§. The logs of daily activities provided by participants were used to identify day (awake) time and sleep time. The recording portions corresponding to intervals where the appliance was not worn (e.g. meal time and shower) were identified and removed. The mean, standard deviation and the coefficient of variations (standard deviation/mean × 100) were calculated, and the results are presented in Table 1.

The reliability of measurement was assessed by intraclass correlation coefficient (ICC). Comparisons of means were performed using Student's paired *t*-test. Statistical analyses were performed using SPSS software (Release 22.0¶¶¶¶).

Table 1. Summary of intra-oral pH and temperature over 24 h of six subjects separated into day time and night time

	During day time: awake (s.d.)	During night time: sleep (s.d.)
Average intra-oral pH	7·3 (±0·4)	6·6 (±0·4)
Coefficient of variance	5·15%	6·9%
Average intra-oral temperature (°C)	33·9 (±2·5)	35·9 (±0·6)
Coefficient of variance	7·3%	1·6%

§§§§Microsoft Corp., Redmond, WA, USA.

¶¶¶¶SPSS Inc., Chicago, IL, USA.

Results

The results of the *in vitro* accuracy test and the correlation of the glass electrode and pH probe are plotted in Fig. 3. The intra-class correlation coefficient (ICC) between the two measurements was 0·99 (95% confidence interval = 0·97–1·00), which indicates a very good accuracy of the ResTech probe.

For temperature dependence test, the pH readings from the pH electrode increased gradually in a linear fashion with the temperature, when used in pH 7·4 phosphate-buffered saline solutions from 27 to 47 °C and gradually decreased in a similar fashion from 47 to 27 °C. At 37 °C, the pH electrode was found to give an accurate reading of pH 7·4. With a 10 °C increase in temperature, the pH reading from the Restech probe increased by pH 0·37, whereas it decrease by pH 0·37 with a 10 °C decrease in temperature, thus indicating a temperature gradient of 0·037 pH/°C.

The readings from the Restech pH electrode after the temperature compensation (mean pH 7·4 ± 0·05) showed a good consistency with that from the gold standard glass electrode (mean pH 7·4 ± 0·01), and there was no significant difference between the two (*P* value = 0·23).

Mean intra-oral pH and temperature values obtained during the two recording days were very similar, the differences being not statistically significant ($P \geq 0{\cdot}14$). The mean pH value during the daytime was 7·3 ± 0·4 for the first recording session and 7·3 ± 0·2 for the second recording session (*P* = 0·97), whereas the mean pH during sleep time was 6·6 ± 0·4 for the first recording session and 6·6 ± 0·2 for the second recording session (*P* = 0·84). The mean intra-oral temperature during daytime was 33·9 ± 0·9 for the first recording session and 33·0 ± 1·2 (*P* = 0·14), whereas the mean temperature during sleep time was 35·9 ± 0·5 for the first recording session and 36·0 ± 0·4 for the second recording session (*P* = 0·38). For convenience purposes, only data from the first recording session were analysed further.

The average salivary flow rate of participants was 1·02 mL min^{-1} (±0·05), which confirmed that all participants were healthy saliva secretors. The examples of pH and temperature data of each individual are presented in Fig. 4, which show differences in fluctuation patterns of intra-oral pH and temperature. During sleep, a slight drop in mean pH (−0·7; 95%

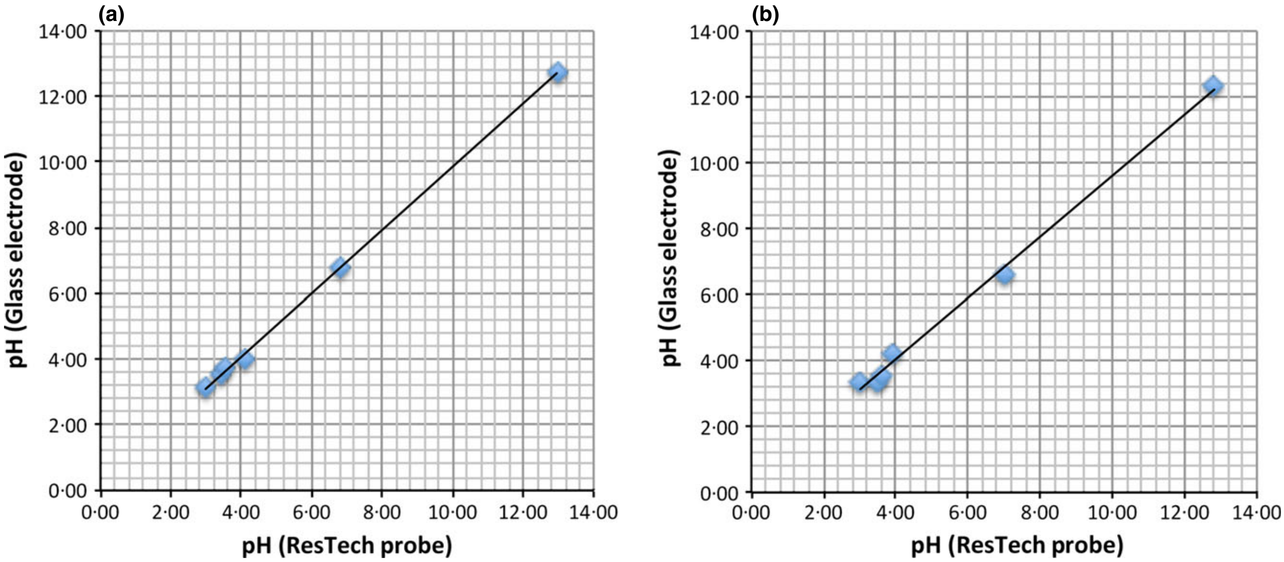

Fig. 3. Shows scatter plot of pH readings of six solutions obtained by ResTech probe compared with a glass electrode used as gold standard. (a) Test 1 (b) Test 2.

CI = −0·4, −1·0) was observed for all subjects. Moreover, intra-oral pH fluctuated more during sleep (CoV of 6·9%) compared to when subjects were awake (CoV of 5·15%). The opposite trend was observed for intra-oral temperature, as reflected in coefficient of variation values (Table 1). The intra-oral temperature was slightly but significantly higher during night than during day (+2·0 °C; 95%CI = +0·6 °C, +3·3 °C). Such difference may most likely be due to jaw functional activity including speaking and the consumption of cold drinks during daytime.

Discussion

The Restech Dx-pH measurement system is an ambulatory pH monitoring system, which was originally developed to measure oropharyngeal pH and to monitor gastroesophageal reflux. Here, we modified this system for intra-oral use, with the addition of a thermocouple to measure intra-oral temperature at the same time. Our *in vitro* findings support the validity of accuracy and the temperature dependence of Restech probe to measure intra-oral pH as compared to a gold standard glass pH electrode. Our *in vivo* findings support its successful use and application as a reliable intra-oral pH monitor.

Our device has various novel features. The small-sized measuring probe, application of soft bleaching tray material and minimal coverage of the dentition, occlusion and the hard palate reduce the possible irritation and stimulating effect in the mouth, allowing the subjects to wear the device comfortably for 24 h. Previous studies (14, 15) argued that the tube connecting the intra-oral appliance and the transmitter may interfere with one's daily activities. However, the volunteers reported that no particular displeasure was felt while wearing the device except its occasional visual presentation in public.

Gudmundsson *et al.* (11) measured the intra-oral pH for 24 h in a similar manner as in the current study. Their results, however, were in contrast to ours; we observed a distinctive change in pH between daytime and during sleep, whereas that of Gudmundsson *et al.* (11) presented a stable pH level throughout the same period. This may be due to the different intra-oral sites where the pH was measured and emphasises the need to carry out further studies. The easy construction, placement and retrieval of the probe and thermocouple of our appliance make the measurement at various intra-oral locations feasible also in large sample size.

Moore *et al.* (17) measured the intra-oral temperature for 24 h using a thermocouple and their results were consistent with ours, with similar ranges and patterns of intra-oral temperature variation over time. They also found no significant difference in temperature change during day and night (during sleep), as observed in the current study.

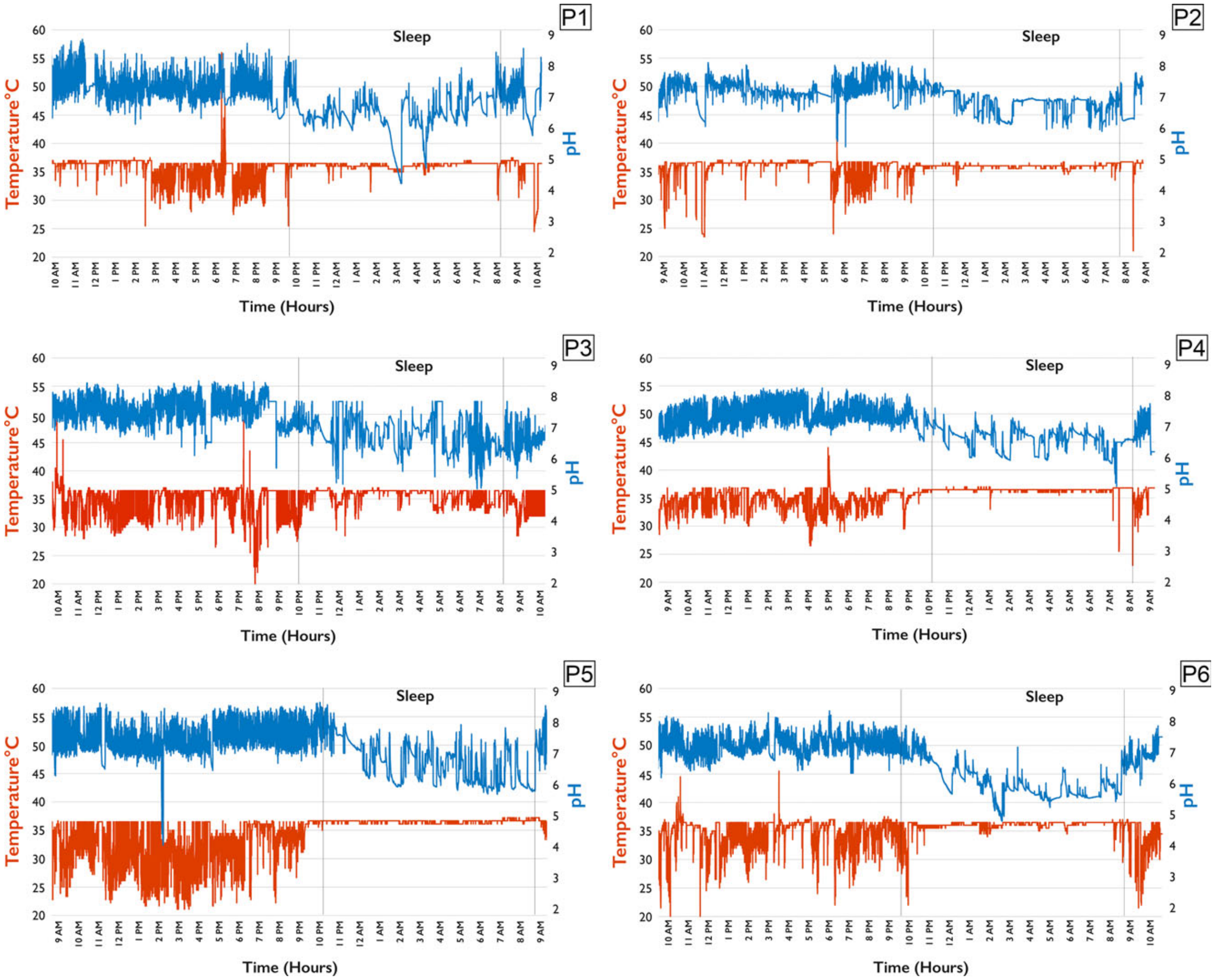

Fig. 4. Intra-oral pH and temperature over 24 h (1st study day) of each individual subject. For each graph: the y-axis to the left indicates temperature; the y-axis to the right indicates pH; the x-axis indicates time. Upper line indicates pH; the lower line indicates temperature

Clearly, the continuous intra-oral pH and temperature measuring with a simple device has further applications. Firstly, it can be used to monitor the change in pH and temperature after consumption of various acidic beverages to investigate their erosive potentials. Despite a number of studies have recorded the erosive potential of food and beverages (8, 10), none of them have investigated the long-term effect, as the in-mouth recordings were only limited to a few minutes after exposure. Continuous and long-term measurement will enable the monitoring of pH change in relation to buffering capacity of saliva and its effect during the hours after beverage intake. Secondly, being able to successfully describe the difference between the pH and temperature variations in normal breathers and open mouth breathers, the device can be used as a diagnostic tool for individuals with oral health issues related to respiratory disorders.

The foregoing emphasises the clinical significance of long-term intra-oral pH in real-life settings. Along with a large body of studies reporting the increased prevalence in dental erosion due to extrinsic and intrinsic factors, a number of recent studies report the fall in intra-oral pH and consequential rise in prevalence of dental erosion and caries among patients with saliva dysfunctions (19, 20), respiratory disorders (21) and association of these disorders with sleep (22–24). However, there are only few studies investigating the relationships between these factors. Furthermore, the monitoring of intra-oral pH and temperature in individuals with and without oral health issues during the day and night will provide normative data, and new insights into inter-individual variation and circadian rhythm, which has not been investigated fully.

Conclusion

The continuous and simultaneous intra-oral pH and temperature measurement system presented in this report is reliable, easy to construct, able to measure variables over a sustained period and may serve as a future diagnostic tool in a number of applications.

Ethical approvals

This study has been approved by the University of Otago Human Ethics Committee (H14/012).

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Conflict of interest

The authors declare no conflicts of interest.

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