Chemistry Africa (2019) 2:301–307
https://doi.org/10.1007/s42250-019-00050-5

ORIGINAL ARTICLE

Extrinsic Fiber-Optic Sensor for Detection of Saliva pH

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Received: 20 December 2018 / Accepted: 5 February 2019 / Published online: 20 February 2019

Abstract
Measurements of pH are important in industry, agriculture, medicine, etc. Two main principles, namely electrochemical and optical ones, have been employed in pH meters and sensors. This paper is aimed at the development of extrinsic fiber-optic sensors of saliva pH. Such sensors have already been used for monitoring pH changes caused by biological processes. In this paper, fiber-optic sensors consisted of inlet and outlet silica fibers transmitting light from a halogen lamp to a sample cell and then to a diode-array spectrometer. Two types of sample cells were used; namely a silica cell with a measurement path of 10 mm, and a special silica capillary cell with a hole diameter of 0.07 mm and measurement path up to 40 mm. This capillary, produced at the Institute, consists of a Bragg mirror applied onto the inner silica capillary wall. Both the sensors were calibrated by using Sorensen buffers with bromothymol blue and a commercial pH meter. The calibration curves were used for the determination of pH of saliva samples collected from one healthy person at different times. It has been found that the fiber-optic sensors provide us with lower pH values than the pH meter. This result can be explain by effects of saliva components on bromothymol blue spectra. By using a correlation line between pH values measured by the sensors and those from the pH meter the sensor reliability of about 0.3 pH units can be estimated.

Keywords Fiber-optic sensor · Rectangular cell · Bragg capillary cell · Sorensen buffer · Bromothymol blue · Saliva pH

1 Introduction

Measurements of pH are important in industry, agriculture, medicine, etc. [1]. It is well known that pH substantially influences microscopic structure and physiological function of many proteins and thus controls physiological processes in cells and tissues [2]. The knowledge of such processes is important for medicine. Results of medical studies have been published which relayed on monitoring of esophageal pH [3], venous and arterial blood pH [4], etc. These studies have shown that the monitoring of pH of body fluids, such as urine, blood, saliva, allows us to detect deviations from the body acid–base balance which can indicate some pathological processes.

In medicine, pH measurements are usually carried out on samples of blood or urine. However, saliva represents a viable alternative to blood sampling [5, 6]. Saliva is composed of water (more than 95%), and of various electrolytes, hormones, enzymes, immunoglobulins, cytokines, etc. [7]. It works as a buffer with reported buffering capacities of 2–4 [6, 8].

Two main principles, namely electrochemical and optical ones, have been employed for pH measurements [1]. Such measuring principles have also been implemented in sensors [9]. Mainly electrochemical measurement methods and sensors have been used for measuring pH of saliva samples [3, 4, 6, 8–10]. Optical methods and sensors have been less employed for saliva pH determination. A litmus paper has been used to assess values of saliva pH in a study of the risk of dental caries in children [11]. An extrinsic fiber-optic sensor has been developed for the investigation of the interaction of saliva with sucrose. This sensor relayed on

Paper based on an Invited Lecture presented at MADICA 2018 Conference (Matériaux et Applications aux Dispositifs et Capteurs), 7–8 November 2018, Mahdia, Tunisia.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s42250-019-00050-5) contains supplementary material, which is available to authorized users.

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spectral changes of bromothymol blue in a wavelength range of 450–650 nm [12]. It has been found that the temporal sensor response at a wavelength of 595 nm well correlate with pH changes measured by an electrochemical pH meter. An evanescent-wave fiber-optic sensors has been investigated at which a pH indicator bromophenol blue was immobilized in a porous membrane in the detection site [13]. This sensor has been used for assessing effects of mutants *Streptococci* onto saliva samples containing sucrose. Temporal changes of the sensor output at 597 nm correlated with a decrease of pH measured by an electronic pH device. Another optical sensor for measuring saliva pH is based on localized surface plasmons (LSPR) of gold nanoplates applied onto a transparent glass slides together with polyaniline [14]. By measuring absorption spectra of such slides in a spectral range from 400 to 1000 nm it has been found that these spectra change with pH. Although the response of this sensor to pH changes has been observed at a wavelength of 795 nm, such a sensor has not been used for pH measurements of saliva pH.

From above paragraph on optical saliva sensors one can conclude that such sensors have been employed for monitoring pH changes caused by saliva samples and not for the determination of pH of saliva samples. This paper presents two extrinsic fiber-optic sensors for pH measurements on saliva samples. Two types of sample cells were used; namely a silica spectrometric cell with a path length of 10 mm, and a special glass capillary with a hole diameter of 0.07 mm and path length up to 40 mm. These sensors have been calibrated by using Sorensen buffers and an electronic pH meter. Results of the sensors obtained on real saliva samples are compared with those determined by the pH meter.

2 Experimental

2.1 Experimental Sensor Setup

A principal setup of the extrinsic fiber-optic sensors used is shown in Fig. 1. It consists of a halogen lamp HL-200 (Ocean Optics), inlet polymer-clad silica (PCS) fiber with a core diameter of 0.3 mm and length of about 1 m, sample cell, outlet PCS fiber with a core diameter of 0.2 mm and length of about 20 cm, and a diode array spectrometer USB 650 Red Tide (a spectral range 350–1000 nm, wavelength resolution 1 nm). The inlet and outlet fibers had the numerical aperture of about 0.4 and they were provided with optical connectors. These connectors allowed us to connect the fibers with the halogen lamp and the spectrometer as well as to set the inlet fiber in line with the outlet one.

Two types of the sample cell were used. First one was a standard rectangular silica cell with a path length of 10 mm. Second cell consisted of a silica capillary modified on the inner wall by a reflecting Bragg mirror (see a photo in Fig. 2). The capillary had a hole diameter of 0.07 mm and a length of 50 mm. Such a capillary enabled us to decrease a sample volume and improve light guiding through the capillary hole. It was prepared at the Institute of Photonics and Electronics by using the Modified Chemical Vapor Deposition Method (MCVD). In the preparation, a multilayer coating consisting of three pairs of alternating layers of silica and silica doped with germanium dioxide was applied by the MCVD method onto the inner wall of a silica tube [15]. The silica layers were doped with phosphorus pentoxide in concentrations of about 0.5 mol% which allowed us to decrease their deposition temperature to about 1700 °C. The layers of silica doped with germanium dioxide contained of about 20 mol% of GeO_2 and were deposited at temperatures around 1500 °C. Layers with thicknesses of about 10 µm were prepared. Immediately after the application of the last coating layers, the tubular preform was put into a furnace and slowly cooled down to laboratory temperature in order to prevent the formation of cracks in the coating. The preform

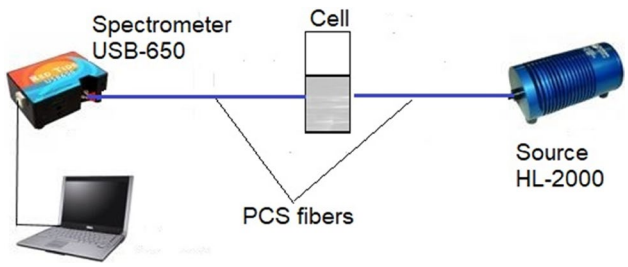

Fig. 1 Scheme of the sensing setup

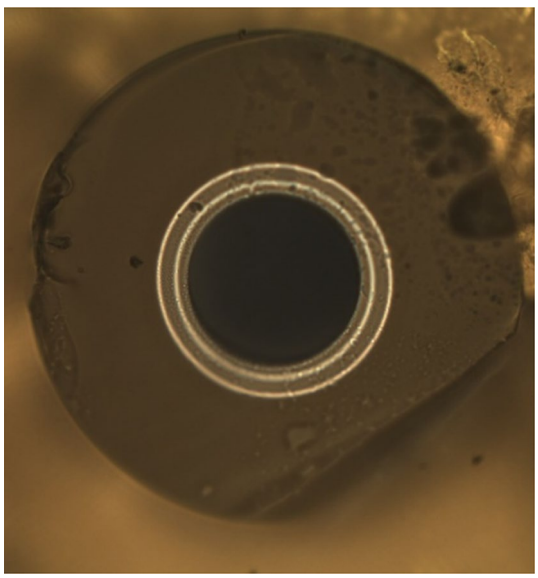

Fig. 2 Optical microscope photo of the cross-section of the capillary cell. Bright parts consist of silica doped with germanium dioxide

was withdrawn into a capillary fiber with a hole diameter of 70 µm and outer diameter of about 200 µm. The fiber was coated with a jacket of UV-curable acrylate (De Sotto) that protected the fiber surface against humidity access. During the withdrawing, a reflecting Bragg mirror with high-index layers of silica doped with germanium dioxide (see the bright parts in Fig. 2) and low-index layers of silica was formed from the preform coating due to viscous flows.

2.2 Calibration solutions and pH measurements

Sorensen buffer solutions were prepared for the calibration of the fiber-optic sensors. They were mixed of stock solutions of 0.0667 M KH_2PO_4 and 0.0667 M Na_2HPO_4. All chemicals used were supplied by Sigma-Aldrich, CR. Characteristics of these solutions are shown in Table 1. For the pH calculations a calculator reported elsewhere [16] was employed.

An electrochemical pH meter Hanna HI8424 (Hanna Instruments) with a probe consisting of an ion selective pH electrode and a reference silver chloride electrode was used. The probe was calibrated by using commercially supplied buffers (Sigma-Aldrich, CR) with pH of 4.01, 7.01, and 10.01.

Table 1 Characteristics of Sorensen buffer solutions used for the sensor calibrations

n (KH_2PO_4)/n (Na_2HPO_4)	pH calculated	pH measured	Ionic strength [mmol/l]
19	5.59	5.53	74
9	5.91	5.86	80
4	6.24	6.23	92
1.5	6.64	6.67	118
0.67	6.98	7.03	145
0.25	7.38	7.44	172
0.053	8.04	8.09	192

In Table 1, n (KH_2PO_4)/n (Na_2HPO_4) denotes a ratio of molar amounts of KH_2PO_4 and Na_2HPO_4 in each Sorensen buffer used

2.3 Saliva Samples

Saliva samples in volumes of about 3 ml were collected from one healthy person by spiting into a sterile plastic cups. Their description is reported in Table 2.

Two measurements were usually used for saliva sample characterizations. Second measurement was carried out at about 30 min after 1st one. Some saliva samples were filtrated through a filter paper Grade 1.

3 Results and Discussion

3.1 Sensor Setup

A principal setup of the intrinsic fiber-optic sensor investigated in this paper differs a little from that described elsewhere [12]. One difference consists in different types of optical fibers employed for guiding light to and from the cell. In our setup, step-index PCS fibers were used which exhibit a higher numerical aperture than commercially available graded fibers reported elsewhere [12]. An increased numerical aperture enables better acquisition of light from a sample cell.

Moreover, a special capillary cell was tested in this paper. It consists of a silica capillary with a reflection mirror applied onto the inner capillary wall. In fact, glass capillaries have been used for different photonics applications including sensors [17, 18]. In such a capillary optical modes are guided in the capillary wall and their evanescent parts penetrate into the capillary hall in which analytes are filed-in. However, the capillary cell used in this paper is similar to a hollow-core Bragg fiber (HCBF). In such a fiber light is guided in the hollow-core due to the photonic band gap of a Bragg mirror applied onto the inner capillary wall. HCBFs have been tested for refractive-index [19, 20], and absorption-based sensing [21].

A hollow-core optical Bragg fiber with the cross-section shown in Fig. 2 was used as the capillary cell in this paper. It is necessary to admit that this fiber exhibit minimum optical

Table 2 Description of saliva samples

Saliva sample	Time of saliva sample collection	Sample description
I	Collected before lunch	Un-filtrated and filtrated samples measured within 30 min after the collection
II	Collected after waking up	Measured 3 h after the collection
III	Collected after waking up and drinking 0.5 l of water	Measured 3 h after the collection
IV	Collected 1 h before lunch	Un-filtrated and filtrated samples measured within 30 min after the collection
V	Collected 1 h after lunch	Measured within 30 min after the collection
VI	Collected 1 h after lunch	Measured within 30 min after the collection
VII	Collected 2 h after lunch	Measured within 30 min after the collection

losses at a wavelength of 1064 nm [15] and not in a wavelength range of 400–700 nm at which bromothymol blue has its absorption bands. However, in this range one can identify several higher leakage modes propagating in the capillary hole [22] and thus, such a fiber can be considered as an ARROW waveguide and used for absorption-based sensing. Provided that the capillary cell with a length of about 4 cm is used, it allows us to work with volumes of measured solution of about 0.15 µl which is much less than of about 0.5–3 ml used in a standard cell with a path of 1 cm. Due to the longer path of the capillary cell, higher absorption changes can be measured with the capillary cell.

In this paper the extrinsic fiber-optic sensors have been tested for pH measurements and not only for monitoring pH changes caused by biochemical process [12, 13]. For such measurements, the sensors were calibrated by using the electrical pH meter Hanna and Sorensen buffer solutions. Then, these calibrations were used for measurements of saliva sample's pH. As both the sensors represent a one-beam spectrometer, a reference measurement was necessary to determine spectral changes of bromothymol blue pH indicator.

3.2 Measurements with the silica spectrometric cell

At first set of experiments, spectra of the output power I were measured on Sorensen buffers containing bromothymol blue. A reference spectrum I_{ref} was obtained from the measurement with water. For this reference, absorption spectra S of bromothymol blue in Sorensen buffers with different pH were determined on the basis of a ratio I_{ref}/I and they are shown in Fig. 3a. In Fig. 3b, calibration data were determined from heights of the indicator band (a basic one) centered at 620 nm. The line was obtained from a linear fit of the data.

The same reference was used for the determination of absorption spectra of bromothymol blue in a sample Saliva I. A very broad spectrum (see short-dash curve in Fig. 3a) was obtained. As all saliva samples tested were turbid due to the mucus content, such a broad spectrum can be explained by light scattering in the sample that decreases the output power. In order to reduce this turbidity, saliva sample I was filtered through a filter paper Grade I. A clear solution was obtained. The spectrum of this solution is also shown in Fig. 3a (the dash–dot–dot curve). It is very different from the spectrum of the original sample which can be explain by the sample filtering that probably removes some components from the sample. Moreover, the absorption maxima of the acid and basic indicator forms are shifted to longer wavelengths which indicates that the absorption coefficients of these forms are influenced by the saliva composition.

From Fig. 3b, pH values of Saliva I and Saliva I-filtered were determined as 7.06 and 6.38, respectively. As one can see from Table 3, these values differ from those determined by the pH meter Hanna. A large difference has been determined on the filtered Saliva I, which can support the explanation that the filtering removed some substances from this saliva sample.

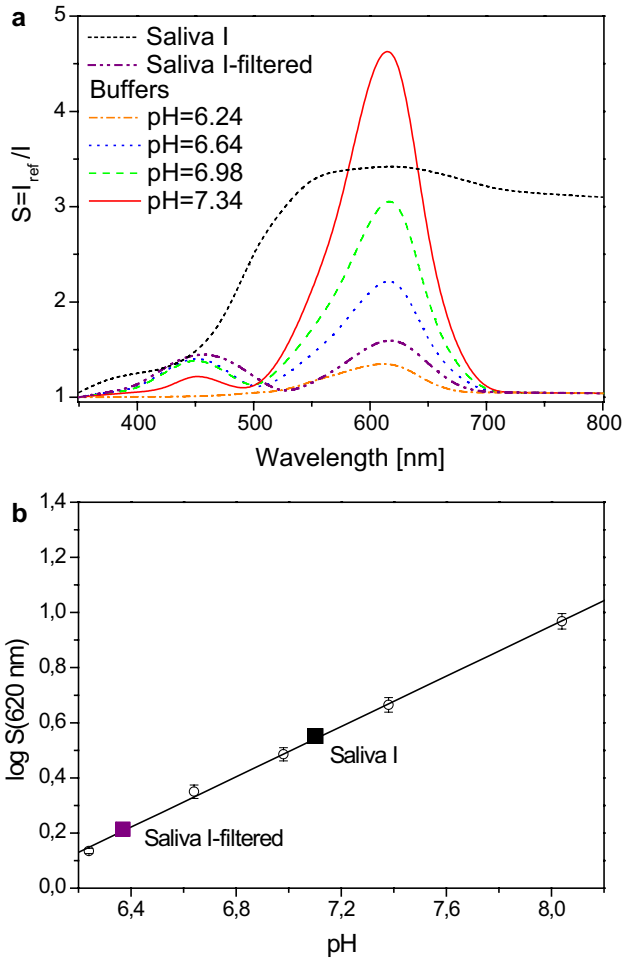

Fig. 3 **a** Absorption spectra of bromothymol blue in Sorensen buffers with different pH and in sample Saliva I measured with the fiber-optic sensor and standard rectangular cell with a path of 1 cm. A reference spectrum of the output power I_{ref} was measured with water. **b** Calibration line of the fiber-optic sensor determined on the basis of measured absorption spectra of bromothymol blue in the Sorensen buffer solutions at 620 nm. The black squares indicate results obtained on samples Saliva I

In order to suppress effects of light scattering in saliva samples, reference measurements were modified. In a novel experimental set, reference spectra of the output power I_{ref} were determined on solutions without bromothymol blue. Absorption spectra S of bromothymol blue with such references are shown in Fig. 4a. Moreover, a novel calibration curve was used that was based on a ratio of values S determined at wavelengths of 460 and 620 nm. It is expected that such a ratio is less dependent on the matrix composition of complex saliva samples than S values at a wavelength of

Table 3 Results of pH measurements on saliva samples

Sample	Sample/sample filtered			
	1st measurement		Repeated measurement	
	pH optical	pH electrical	pH optical	pH electrical
Saliva I	7.06/6.85	6.38/6.98	–	–
Saliva II	6.74/–	6.81/–	6.84/–	6.92/–
Saliva III	6.38/–	6.45/–	6.36/–	6.49/–
Saliva IV	6.77/6.86	6.82/7.01	6.89/6.95	7.14/7.20
Saliva V	6.68/–	7.17/–	6.70/–	7.10/–
Saliva VI	6.65/–	6.70/–	6.59/–	6.75/–
Saliva VII	6.81/–	6.98/–	6.78/–	6.95/–

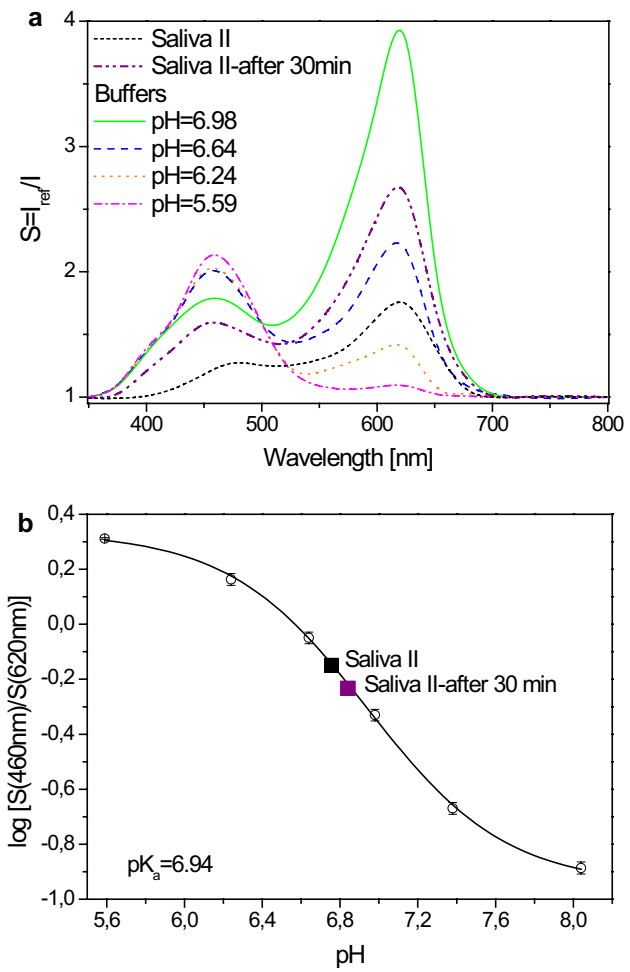

Fig. 4 **a** Absorption spectra of bromothymol blue in Sorensen buffers with different pH and in samples of Saliva II measured with the fiber-optic sensor and standard rectangular cell with a path of 1 cm. Reference spectra of the output power I_{ref} were measured with the buffers and saliva samples without bromothymol blue. **b** Calibration curve of the fiber-optic sensor with the rectangular silica cell determined from measurements of absorption spectra of bromothymol blue in the Sorensen buffers. The squares show results obtained on Saliva II sample

620 nm. The calibration curve in Fig. 4b was obtained by fitting the data with a sigmoidal Boltzmann curve. A value of pK_a of bromothymol blue equal to 6.94 ± 0.02 has been determined. This value agrees well with a tabulated value of about 7.

From Fig. 4b, pH values of 6.74 and 6.84 were determined for Saliva II. The data in Table 3 for this sample show that these values agree well with those of 6.81 and 6.92, respectively determined with the pH meter. These values and Table 2 allow us to conclude that there are some small temporal changes even at a saliva sample collected 3 h before the measurements. The absorption spectra of bromothymol blue in Saliva II in Fig. 4a are different for both the measurements although the pH values differ by about 0.1 pH unit. They show that there are some effects of saliva onto the absorption spectra of bromothymol blue. Similar spectra have been measured on a saliva sample by using a spectrometer Perkin Elmer Lambda 365 UV–VIS and a standard cell with a path of 1 cm (see Supplementary Material S1).

By using the novel reference measurements and calibration curve, pH values of another saliva samples were determined by using the sensor with the silica cell. Measurements with the pH meter were also carried out on the same samples. These values are summarized at Table 3. By plotting pH values obtained by the pH meter for samples Saliva II–Saliva IV against those determined by the sensor, Fig. 5 is obtained. From this figure one can find that pH values determined by the sensor are lower than those measured by the pH meter, namely for pH values higher than of about 6.7. This difference can be explained by ionic strengths of saliva samples that can range from 20 to 200 mM as calculated from contents of salts in saliva [7]. The ionic strength

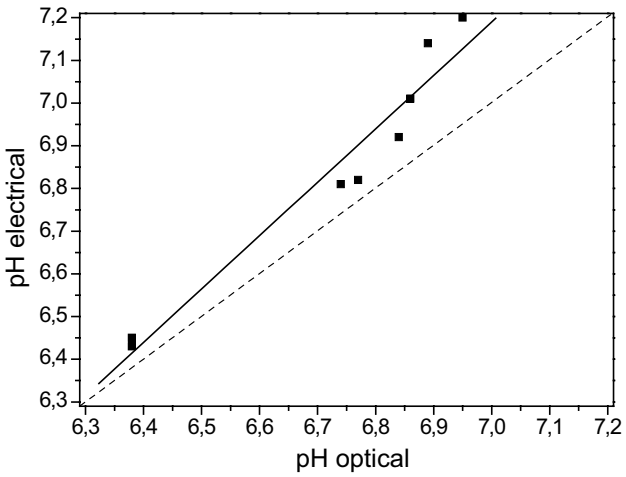

Fig. 5 Correlation between pH values of saliva samples determined by the fiber-optic sensor with the silica cell and those from the pH meter. Solid line represents the correlation of both the measurements and dashed one identical pH values

influences the apparent K_a of bromothymol blue and thus the content of the basic and acidic forms. However, there is another explanation that consists at different values of light absorption coefficients of bromothymol blue in Sorensen buffers and in saliva samples. One can support this explanation by different isosbestic points of bromothymol blue in saliva and in buffers (see Fig. 4a).

By making a linear fit of data in Fig. 5, a correlation line of optical and electronic pH values is obtained (see the solid line in Fig. 5). This line is characterized by a value of $R^2 = 0.97$. The line allows us to correct pH values determined by the sensor into expected values obtained by the pH meter. Such corrections have been tested on Saliva V. With this sample, pH values of 6.68 and 6.70 were determined by using the sensor. Corrected values of pH are 6.79 and 6.82, respectively. As one can see from Table 3, pH values measured for Saliva V by the pH meter are higher by about 0.3 pH units than the corrected ones. This difference can be interpreted as the reliability of this fiber-optic sensor for higher pH values. Similar differences have been found for an electrical sensor [10].

3.3 Measurements with the Capillary Cell

Absorption spectra of bromothymol blue determined by using the sensor with the capillary cell are shown in Fig. 6a. A column of a measured sample with a length of about 2.5–3 cm was soaked into the cell at these experiments. Spectra of the output power of the samples without bromothymol blue were taken as the references. A calibration curve in Fig. 6b is similar to that in Fig. 4b and takes into account a ratio of relative output powers at 460 and 620 nm.

From the calibration curve in Fig. 6b, pH values of the saliva samples VI were determined (see Table 3) which were corrected by using the correlation line in Fig. 5b. Corrected values of 6.78 and 6.70 agree well with those of 6.69 and 6.75, respectively determined by the pH meter. Another measurements with the capillary cell on Saliva VII provided us with pH values of 6.91 and 6.78 (see Table 3). Corrected values of 6.92 and 6.88 are close to those of 6.98 and 6.95, respectively measured by the pH meter.

3.4 Comparison of Performance of Both the Tested Cells

Results presented in Table 3 show that both the sensor with the standard rectangular cell and that with the capillary cell exhibit the similar performance for pH measurements on saliva samples. The tested sensors have allowed us to determine reliable pH values for to the high saliva acidity range, i.e. for pH values lower than 6.2 [23]. An opened issue for these optical sensors is the determination of reliable pH values when saliva pH is higher than 7.5. For such purpose,

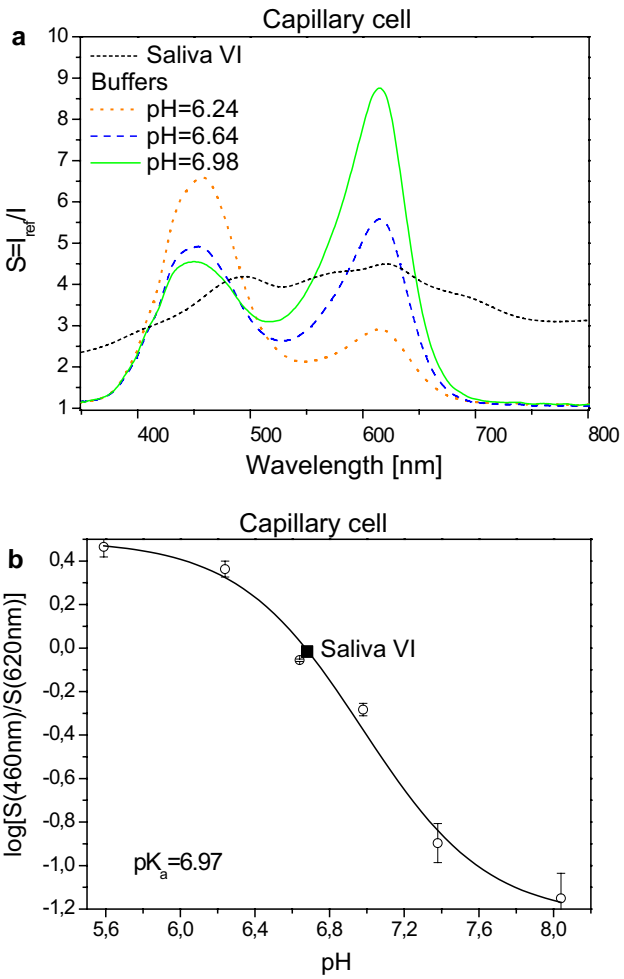

Fig. 6 **a** Absorption spectra of bromothymol blue in Sorensen buffers with different pH and in sample Saliva VI measured with the fiber-optic sensor and capillary cell. Reference spectra of the output power I_{ref} were measured with the buffers and saliva samples without bromothymol blue. **b** Calibration line of the fiber-optic sensor with the capillary cell determined from absorption spectra measurements on Sorensen buffer solutions. The square indicates results obtained on Saliva VI sample

pH calibration solutions based e.g. on artificial saliva can be employed for measuring more realistic spectra of bromothymol blue.

Some differences at characteristics of both the sensors tested have been observed. Thus, the rectangular cell exhibits advantages such as the precise optical length and easy filling with liquid samples. A minimum sample volume of about 0.5 ml is necessary for the sensor with such a cell, which is not limiting for measurements on body fluids. A maximum sensitivity of about 0.90 $(\text{pH unit})^{-1}$ can be estimated from Fig. 4b.

The capillary cell requires very small sample volumes of about 0.15 µl. Such a cell allows us to increase detection length to several centimeters which enables to increase the

sensors response and sensitivity. A maximum sensitivity of about 1.29 (pH unit)$^{-1}$ was estimated from Fig. 6b. However, the reproducible filling such a capillary with viscous fluids is difficult. This filling influences cell characteristics as the length and shape of the sample column in the capillary and its excitation. One can expect that these characteristics could be controlled by using sophisticated microfluidic setups [24].

4 Conclusions

It has been shown that extrinsic fiber-optic sensors calibrated by using an electrochemical pH meter and Sorensen buffers with bromothymol blue can be employed for measuring pH values of saliva samples with the reliability of about 0.3 pH units. This difference is explained by effects of the saliva composition on the absorption spectra of bromothymol blue in saliva samples. Thus, such measurements can be reliably used for monitoring changes of saliva sample pH changes rather than for precise pH measurements of saliva.

A standard rectangular silica cell and capillary cell can be used with extrinsic fiber-optic sensors. Such cells allow us to decrease measured saliva volumes to about 0.5 and 0.00015 ml for the standard and capillary cells, respectively. While commercial setups are available for precise measurements with rectangular cells some technical issues, such as filling in samples into the capillary and its excitation have to be solved for capillary cells, e.g. by using microfluidics. Measurements of pH with intrinsic fiber-optic calibrated on buffers based on artificial saliva are planned for future experiments.

Acknowledgements This work was financially supported by the Czech Science Foundation (contract 16-10019S).

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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