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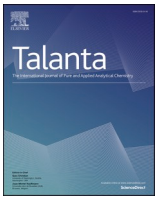

Plasmonic sensor for hydrogen sulphide in saliva: Multisensor platform and bag format

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ABSTRACT

In recent years, there is a growing demand for optical sensors given their analytical properties, and the possibility of in situ implementation. Among all the types of optical sensors, plasmonic sensors have aroused great interest in the scientific community. In this work, the ability of a plasmonic sensor based on AgNPs retained on a Nylon surface is studied to determine hydrogen sulfide, which can be an indicator of oral diseases. This compound produces a color change of the sensor from yellow to brown directly related to its concentration. The sensor response is evaluated in two different assay formats such as bag and well microplates. The figures of merits of both methodologies have been obtained and compared. The advantages and disadvantages of the different formats have been shown. Finally, the sensor is applied to quantify sulfides in real saliva. Concentrations ranged from 30 μ L^{-1} to 600 μ L^{-1} have been obtained for the voluntaries. Besides that, in this approach the RGB coordinates from images have been used as analytical signal too. The results achieved have demonstrated that the sensor and the methodology applied provide good selectivity, sensibility, rapidity, it is non-invasive and it can be used as indirect method to measure problems in the oral cavity.

1. Introduction

Through the years, an exponential increase has been done on research and development of optical sensors. For example, in 2000, annual publications about this type of sensors were about 3000 per year but in 2021 the amount of works increased until 11,000 (Scopus Database, consulted in December 2021 using as keywords optical and sensors). Moreover, optical sensors have a great potential in applications on different science areas such as biotechnology, biomedicine, molecular biology, or chemistry. In addition, detection time, reusability, precision, and other requirements of this type of sensors can be adapted to the characteristics and concentration of the analyte in the sample [1].

Among all known nanoparticles, silver (AgNPs) and gold (AuNPs) are generally the most used, as these two metals exhibit optical, electrical and photothermal properties that make them very interesting elements for sensors [2]. AgNPs represent an important innovation in the field of nanotechnology. Due to this, there is a growth in the demand to produce this type of nanoparticles [3]. Silver nanoparticles have numerous applications in many fields, such as environmental chemistry, agricultural sector, biomedicine, textile industry, among others [4].

In recent years, saliva has received increasing attention as an alternative for disease detection [5,6]. Sample collection is simple, does not require specialized personnel and can be performed by the patients themselves. Sampling is quick and inexpensive, and saliva storage is simpler than that proposed for many conventional matrices [6–9]. Regarding its composition, it varies during the day and between individuals. When expressing saliva constituents and their concentrations, average compositions found in healthy subjects are usually used. In general, saliva is composed of 99% water, with the remaining content consisting of a variety of ions including sodium, potassium, calcium, bicarbonate, thiocyanate, and phosphate, which constitute 0.2% of total saliva. Low molecular weight organic substances such as uric acid or lactate, immunoglobulins, enzymes and mucus have also been found in saliva [10–15].

Within all applications, there are not many applications in which AgNPS have been used in saliva analysis. Alzahrani et al. [16] synthesized silver nanoparticles from durian fruit shell and they were used as an optical sensor for ammonia determination with colorimetric

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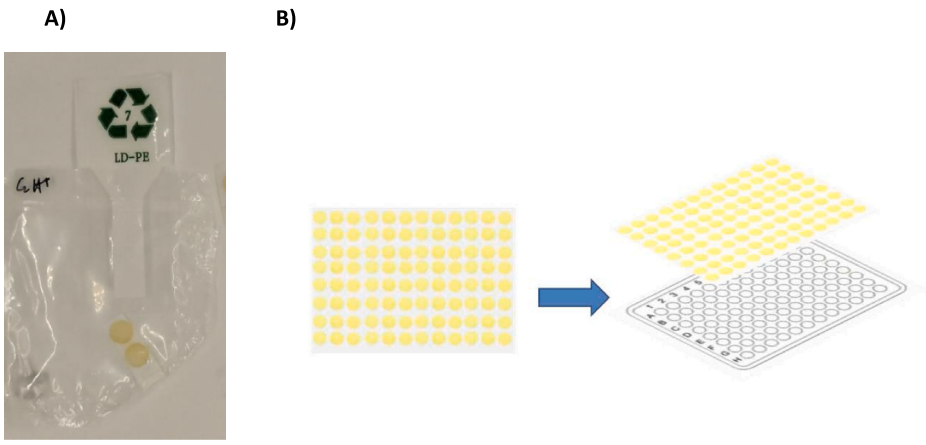

Fig. 1. Saliva analysis format A) bags B) Nylon sheets to be adapted to microplate.

detection. Sheini et al. [17] developed a paper-based devices using thiomalic acid capped with silver nanoparticles for ammonia detection and maltol capped for carbon dioxide detection.

Oral diseases such as periodontitis and gingivitis are chronic inflammatory conditions that may affect as much as 80% of the adult population. This disease is caused by the accumulation of bacteria and produces halitosis or bad oral breath [18]. The main cause of bad odor is the degradation of sulfur-containing amino acids produced by anaerobic bacteria, which produce so-called volatile sulfur compounds (VSCs) [19,20]. The determination of VSCs is of interest in many fields. Within the VSCs, one of the most monitored gases is hydrogen sulfide (H_2S) [21], although there are also different compounds that present sulfur, such as methyl mercaptan (CH_4S) and dimethyl sulfide (C_2H_6S) that are of equal importance, although they tend to occur in less concentration. More specifically, in the case of halitosis, H_2S, C_2H_6S and CH_4S constitute 90% of the characteristic VSCs of this disease [22,23].

H_2S determination has some difficulties because of the instability of H_2S, its high volatility and rapid oxidation. Thus, there are controversial results about the found concentrations. The extraction and sample pretreatment of H_2S may face interference, and minimal sample treatment is recommended. Considering that H_2S is a very volatile compound, its concentration may decrease during long sample processing. From the analytical point of view there is the need of sensitive, selective, rapid response and non-invasive methods for quantifying H_2S in vivo and in vitro [24].

When determining this type of compound, the most widely used analytical technique is gas chromatography (GC) [25,26]. However, other methods. Such as electrochemical analysis [27], colorimetric [28], fluorescent methods [29] and metal nanoparticle sensors [30] have been used to measure H_2S in different matrices. Among the different methods of detection and quantification of these compounds, Campíns-Falcó et al. [31] patented a plasmonic sensor that retains AgNPs on a nylon membrane and it has been proposed for the determination of H_2S in exhaled samples. In Ref. [21] it was demonstrated that the presence of VSC produces an aggregation reaction of AgNPs retained on nylon and then, plasmonic band shifts to higher wavelengths. The presence of VSC produces an aggregation reaction of the nanoparticles that results in a color change of the sensor from yellow to brown depending on the concentration. The low sensitivity and the low concentrations of CH_3SH and $(CH_3)_2S$ compared to H_2S will allow to determine H_2S selectively. Different compounds, that can be found in the oral cavity, such as ammonia, acetone, or ethanol, among others, were tested and they did not provide significative sensor response [21].

Saliva is believed to be one of the main sources of oral malodor because it contains a large amount of sulfur-containing substrates that can be hydrolyzed and degraded to volatile sulfur compounds. Not many articles have been published concerning to sulfur determination in saliva. Quirynene et al. have proposed a salivary incubation test for evaluation the bad olor [32], that required between 3 and 6 h. E. Zaorska et al. [33] proposed a fluorescent probe for determination of H_2S also in saliva samples. These authors found H_2S concentrations within a range of 55.8 μg L^{-1}-242 μg L^{-1} in healthy volunteers.

In this work, we aimed to demonstrate the ability of the plasmonic sensor based on the immobilization of AgNPs on a nylon membrane to develop an assay for the determination of H_2S in saliva samples. Due to the selectivity of the procedure, the samples were directly analyzed. Two different formats to carry out the process have been tested, plastic bags and microplate wells. The experimental variables to adapt the test to the expected concentration range and matrix have been studied. The optimized procedures have been applied to determine H_2S in real saliva samples. About the analytical readout, conventional reflectance measurement and RGB coordinates from the images obtained by using a smartphone have been used to calculate the H_2S concentration.

2. Experimental section

2.1. Reagents and materials

Dispersion of Ag nanoparticles of 20 nm, 0.02 mg/mL in aqueous buffer containing sodium citrate as stabilizer, glycerol ≥99% and sodium bicarbonate were provided by Sigma Aldrich (USA). Sodium sulfide was ordered to Scharlau (Australia). Nylon membranes of 0.22 mm pore sized were provided by Filter-Lab (USA). Potassium dihydrogen phosphate, calcium chloride hydrated and phosphoric acid 85% were obtained from Panreac (Spain). Potassium chloride, sodium dihydrogen phosphate and potassium thiocyanate were ordered to Merck (Spain and Germany). Citric acid was from Fluka (Switzerland) and sodium chloride was purchased from Manuel Riesgo (Spain).

Wells microplates were provided by Thermo Fisher Scientific (USA). Vacuum bomb was ordered to KNF (Germany). 250 mL beaker and 2 mL syringe were obtained from Labbox (Spain). 10, 100, 200 and 1000 mL micropipettes and multicanal micropipette were ordered to Labnet International (USA). Nylon filter with 0.45 μm of pore size was purchased from Epica SL (Spain) and a 10 mL volumetric flask were provided from Scharlau (Australia).

2.2. Apparatus

UV–Vis spectra were registered with a Varian Cary 60 UV–Vis spectrophotometer. This instrument is equipped with a diffuse reflectance probe from Harric Scientific Products (Pleasantville, New York, USA), which was used for the diffuse reflectance measurements of the sensor. Photographic images of the sensors were taken with a Samsung A70 smartphone using a "Pro" mode in camera, ISO: 7100 and light/

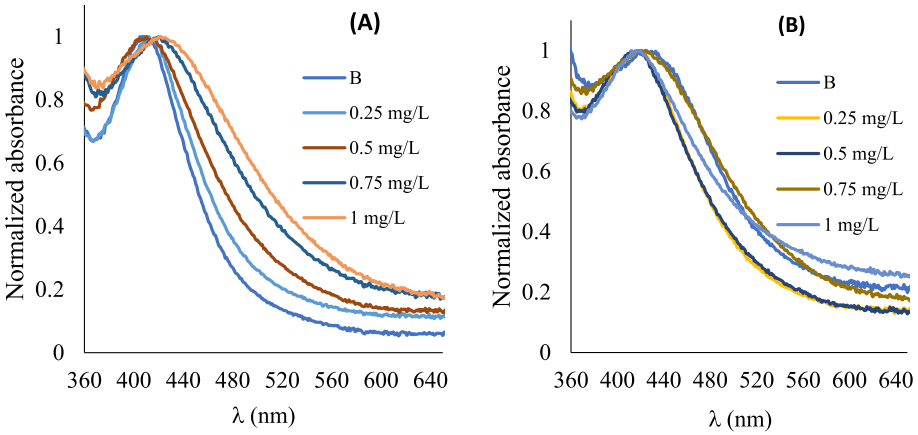

Fig. 2. UV spectra obtained for fortified synthetic saliva solutions in presence (A) and absence (B) of H_3PO_4, for different H_2S concentrations.

contrast ratio of +0.5. Colors were analyzed by free image editor software GIMP (version 2.10.12). The red-green-blue (RGB) color mode was applied to transform the images into numerical color values. Microplate agitation was done with a Microplate Thermostatic Shaker DTS-2 from ELMI (USA).

2.3. Synthesis of AgNPs sensors

In a well microplate, a nylon membrane of 0.22 μm pore size previously cut in a rectangular shape was placed to synthesize the AgNPs sensors. With a multichannel micropipette, 110 μL of water or nanoparticle dispersion, depending on the well, was added to each one. Once all the volumes have been added, the membrane was filtered under vacuum for 5–10 s. The sensors were stored in the dark at 4 °C until used.

2.4. Assays in bags and microplate

In the assays using bags, a pair of sensors and a magnet were introduced on a 5×7 cm bag (Fig. 1A). After that, the bag was sealed and swollen with 30 mL of air and 200 μL of sulfide solution were added. Then, it was stirred for a determined time and, after that, the sensors were removed. 50 μL of glycerol were added to each sensor and reflectance UV–Vis spectra was registered.

Using well microplate, 200 μL of sulfide solution were added to the determined wells. The sensors covered all wells and the microplate was agitated at 550 rpm for a determined time (Fig. 1B). Once analysis is finished, the sensors were removed and 50 μL of glycerol were added to each sensor and reflectance UV–Vis spectra was registered.

2.5. Preparation of synthetic saliva

As a diluent for the sulfide standards and, in order to be able to prepare them in a matrix more similar to that of the real saliva sample, 100 mL of a synthetic saliva solution was prepared according to Marques M.R.C et al. [34]. Once saliva was prepared, it was stored in a refrigerator at 4 °C until use.

2.6. Application to saliva

The whole saliva samples were taken without stimulation and using the spitting procedure. Human saliva samples of volunteers were collected into a 1.5 mL Eppendorf and stored at 4 °C. Prior to analysis, samples were centrifuged at 10,000 rpm for 10 min and acidified with phosphoric acid. For spiked saliva samples, a suitable volume of sulphide solution was added. For filtered saliva samples, a nylon filter with 0.45 μm of pore sized was used.

3. Results and discussion

The sensor developed has been validated according to the standard guidelines of International Conference on Harmonisation ICH [35] and US Pharmacopia [36]. Based on these guidelines, this sensor can be considered an identification test that can determine the amount of H_2S in saliva quantitatively. Thus, besides the selectivity, that is the property to be validated in the identification tests, all the other analytical characteristics have been considered. Typical validation characteristics which have been considered are accuracy, precision, repeatability, intermediate precision, specificity, detection limit, quantitation limit, linearity range and robustness.

3.1. Sampling and pretreatment

There are several parameters that can affect the salivary composition such as age, flow rate, diet, temperature, and pH, among others. Considering that, the results of the saliva analysis depend on the sample taken, in this case the whole samples were taken by using the spitting procedure without stimulation. A normal salivary flow rates above 0.1 mL/min for unstimulated saliva was selected. The samples were taken at room temperature and before eating. Volumes ranged between 0.5 and 1.5 mL were collected and were introduced in an Eppendorf. Samples were centrifuged at 10.000 rpm for 10 min. All the samples analyzed belonged to people between 20 and 30 years. The optimization parameters were stablished by using synthetic saliva (see experimental section).

Previously the influence of the saliva pH was evaluated. Normal saliva pH is between 7 and 7.4 for unstimulated samples and has slight buffer capacity due to the presence of three buffer systems: bicarbonate, phosphate and proteins. pH measurement for a series of saliva samples showed values between 6.40 and 7.20. In saliva, the predominant species at this pH are H_2S and HS^- according to dissociation constants $k_1 = 1 \cdot 10^{-7}$, $k_2 = 1.2 \cdot 10^{-13}$. The sensor responds to the concentration of H_2S,(g) which is related to the total concentration of species in solution.

In view of the pH values in saliva, standards and samples were acidified with H_3PO_4 to obtain a value around 7. To verify the H_3PO_4 influence, two calibrations were carried out, one in presence and the other in absence of H_3PO_4 for a response time of 30 min. Fig. 2 shows diffuse reflectance spectra with (Fig. 2A) and without (Fig. 2B) acid. As it can be seen, acidification produces a shift towards higher wavelengths as would be expected. For this reason, it is advisable to slightly acidify standards and samples to obtain better results.

3.2. Reaction parameters optimization

According to our previous studies, in presence of H_2S, AgNPs

Table 1
Analytical data for wavelength optimization.

Wavelength (nm)	a ± s_a	b ± s_b	R^2	LOD (mg/L)	LOQ (mg/L)
480	0.261 ± 0.008	0.431 ± 0.013	0.997	0.06	0.17
500	0.170 ± 0.011	0.380 ± 0.018	0.994	0.09	0.3
520	0.15 ± 0.02	0.26 ± 0.04	0.942	0.3	0.8
540	0.119 ± 0.019	0.21 ± 0.03	0.937	0.3	0.8
560	0.099 ± 0.016	0.16 ± 0.03	0.928	0.3	0.9

plasmonic band shifts to a higher wavelength. In this sense, different wavelengths were examined to see which of them offered better analytical parameters. For this, wavelengths in the 480–560 nm range were chosen in order to select the one that presented higher sensitivity. Table 1 shows analytical data of the calibration curves for the wavelengths studied. The detection limit (LOD) and quantification limit (LQD) were measured by diffuse reflectance and calculated as 3 Sblank/b and 10 Sblank/b, respectively, where b is the slope of the calibration curve and Sblank is the standard derivation of the intercept. As can be seen, lower detection and quantification limits (LODs and LOQs) were achieved measuring at 480 and 500 nm. Therefore, these two wavelengths were selected for further experiments.

To perform the analysis two different formats were tested, consisting in introducing the sample in: a) a plastic bag containing the sensor b) in a well of microplate well and placing the sensor on the top.

For the bags format, the synthetic saliva samples were acidified in the bags and times of 15 and 30 min of reaction time were studied. It was decided to choose these two times as a shorter time would not be sufficient to fully develop the sensor color and a longer time would unnecessarily increase the analysis time. In Fig. 3A sensors are shown after the exposition to the sample, observing the expected progressive change from yellow to brown. Diffuse reflectance spectra for 15 (Figs. 3B) and 30 (Fig. 3C) minute calibration are also shown. Good linearity was obtained, as well as low detection and quantification limits for the two wavelengths studied at both times (Table 2). In terms of precision, coefficient variation of 2.4 and 15.3%, were obtained for intraday and interday respectively for 15 min and values of 4.1 and 15.6% for 30 min. Due to these results, saliva samples can be analyzed at 15 or 30 min.

Focusing on microplate analysis, the Nylon sheets with AgNPs spots were place on the top of the microplate, each of the spots matched with the hole of the wells. Times of 60 (Fig. 4A), 15 (Figs. 4B) and 5 (Fig. 4C) minutes were studied. Analyzing the results in Table 3 it was observed how the values of the correlation coefficient and sensitivity at 5 min were low, compared to the other two conditions studied, so this analysis time was discarded. Focusing now on 15 and 60 min, it was observed that most of the analytical parameters obtained from the calibration curve were similar. Between these two times, 15 min was finally selected for further experiments.

The next parameter that was tested was the agitation of the microplate. A bigger batochromic shift of the plasmonic band of AgNPs was observed when agitating. Comparing the results shown in Table 3, it is observed that with microplate agitation a higher sensitivity is obtained, as well as lower LODs and LOQs for both 480 and 500 nm. Therefore, it was included the agitation of the well microplate as a part of the procedure.

While using bags, 200 μL of saliva or standard were added [21], but since the wells volume was smaller (400 μL compare 0.5 L), different solution volumes were studied: 100 and 200 μL. Comparing the results, it can see that lower LODs and LOQs are obtained 0.03 and 0.09 mg L^{-1}, respectively, as well as a higher sensitivity, were obtained in the case of using 200 μL (Table 3). For this reason, this final volume was selected for samples analysis. By working at the optimal conditions ((15 min, microplate agitation and 200 μL of solution), the intraday and interday precision were evaluated and values of 0.9 and 11.4% of RSD% respectively were obtained. In order to evaluate the reproducibility, the sensors were tested outside of the laboratory by non-expertise personal and the results of precision were satisfactory with RSD % lower than 12%.

The robustness of the sensor was previously evaluated by Jornet et al. [21] testing the assay at different humidity percentages between 20 and 96% at room temperature (between 20 and 25 °C). No differences in the responses were obtained for humilities between 50 and 96%. The sensitivity decreased for lower humidity (20%). In this case, the figures of merit are shown in Table 3. Concerning to the lineal range, the linearity was evaluated in the range 0.1–1 mg $L.^{-1}$

Table 2
Analytical data for response optimization in bag format.

Wavelength (nm)	Time (min)	a ± s_a	b ± s_b	R^2	LOD (mg/L)	LOQ (mg/L)
480	**15**	0.362 ± 0.005	0.272 ± 0.008	0.998	0.05	0.15
	30	0.261 ± 0.008	0.431 ± 0.013	0.997	0.06	0.17
500	**15**	0.273 ± 0.002	0.246 ± 0.003	0.999	0.019	0.06
	30	0.170 ± 0.011	0.380 ± 0.018	0.994	0.09	0.3

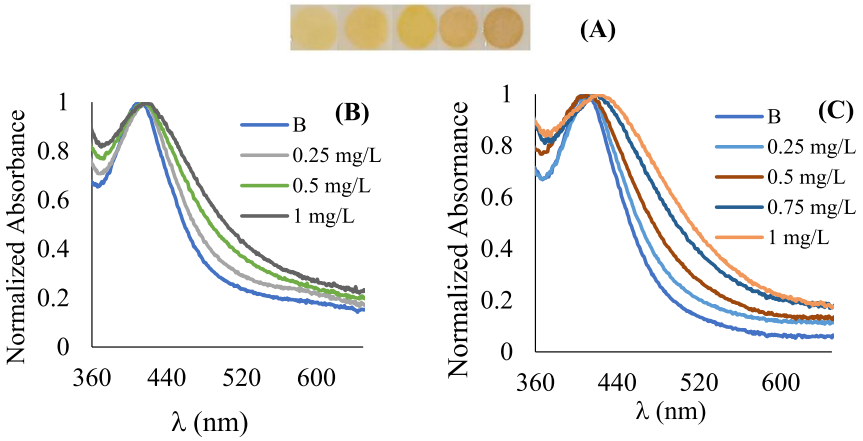

Fig. 3. Exposed sensors to different H_2S concentrations (A). UV spectra obtained for fortified synthetic saliva solutions at different reaction times. 15 min (B) and 30 min (C). Format bags.

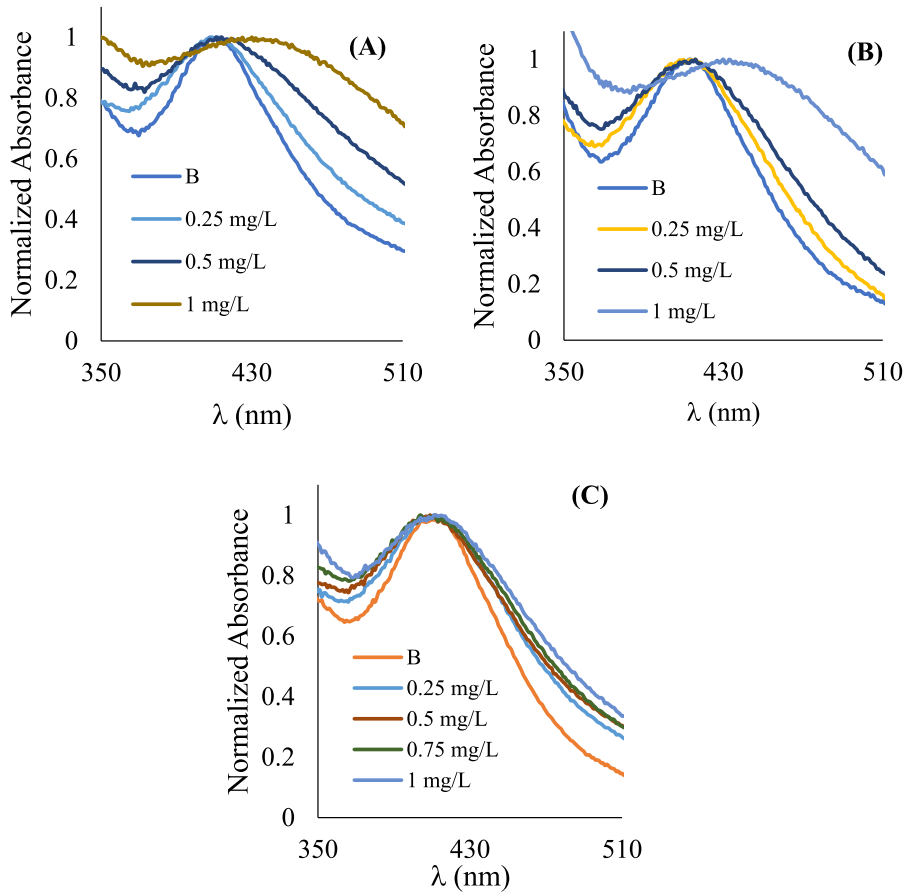

Fig. 4. UV spectra obtained for fortified synthetic saliva solutions at different reaction times. 60 min (A) 15 min (B) 5 min (C). Format microplate.

Table 3
Analytical data for response optimization in well microplate.

Wavelength (nm)		a ± s_a	b ± s_b	R^2	LOD (mg/L)		LOQ (mg/L)
480	**5 min**	0.28 ± 0.02	0.26 ± 0.03	0.956	0.2		0.7
	15min	0.275 ± 0.005	0.537 ± 0.009	0.999	0.03		0.09
	60 min	0.378 ± 0.008	0.493 ± 0.015	0.999	0.05		0.15
500	**5 min**	0.188 ± 0.017	0.23 ± 0.03	0.958	0.2		0.7
	15 min	0.160 ± 0.013	0.52 ± 0.02	0.996	0.07		0.2
	60 min	0.291 ± 0.010	0.480 ± 0.018	0.997	0.07		0.2
480	**No agitation**	0.42 ± 0.03	0.31 ± 0.05	0.945	0.3		0.9
	Agitation	0.275 ± 0.005	0.537 ± 0.009	0.999	0.03		0.09
500	**No agitation**	0.30 ± 0.02	0.27 ± 0.04	0.951	0.3		0.8
	Agitation	0.160 ± 0.013	0.52 ± 0.02	0.997	0.07		0.2
480	**100 µL**	0.249 ± 0.018	0.27 ± 0.03	0.966		0.2	0.6
	200 µL	0.275 ± 0.005	0.537 ± 0.009	0.999		0.03	0.09
500	**100 µL**	0.171 ± 0.012	0.24 ± 0.02	0.986		0.15	0.4
	200 µL	0.160 ± 0.013	0.52 ± 0.02	0.996		0.07	0.2

3.3. Quantification of sulfide in saliva samples

Saliva composition varies between individuals in an unknown way. To reduce this effect as much as possible, it was tried to previously filter saliva after the centrifugation step, using a Nylon filter with a pore size of 0.45 µm. By filtering the bacteria were removed from the samples. Ten unfiltered and filtered saliva samples spiked with 500 µg L^{-1} of Na_2S were analyzed (Fig. 5). As can be seen, three saliva groups can be distinguished with respect to filtration. In the case of saliva 1, 2 and 3 (Group 1), this step does not produce any change in recovery. For saliva 4, 5 and 6 (Group 2), filtration generates a more or less considerable increase in recovery, which indicates that it reduces the matrix effect of the sample. In saliva 7, 8, 9 and 10 (Group 3), filtration leads to a reduction in recovery. In general, matrix effect is observed in all the samples analyzed, so a fortification of the sample should be performed in order to calculate the right recovery. Focusing on well microplate, 10 filtered and 24 unfiltered saliva samples were analyzed at 15 min. The accuracy was evaluated and reported as percent recovery by the assay of known added amount of analyte in the sample. Table 4 shows recoveries for each type of samples at the wavelengths studied. Concentration range was quite wide, having samples with very low recoveries (<30–40%) and others with acceptable ones (80–120%). Comparing the values between the two wavelengths studied, recoveries do not differ significantly from each other. In addition, similar ranges are obtained between filtered and unfiltered saliva. Thus, the presence of bacterium did not affect the responses. Although they are responsible of the

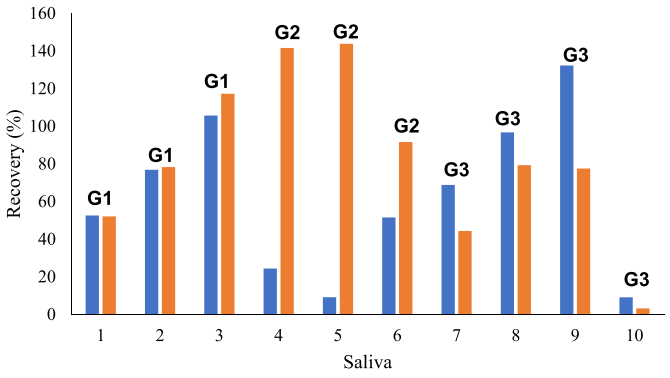

Fig. 5. Recoveries obtained for real saliva samples unfiltered (blue) and filtered (orange). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 4
Recoveries obtained for saliva samples.

		Time (min)	Wavelength (nm)	Recovery (%)
Microwell	Unfiltered Saliva (n = 24)	15 min	480	19–129
			500	17–139
	Filtered Saliva (n = 10)	15 min	480	33–116
Bags	Unfiltered saliva (n = 7)	15 min	480	9–164
			500	11–144
	Unfiltered saliva (n = 3)	30 min	480	63–64
			500	53–70

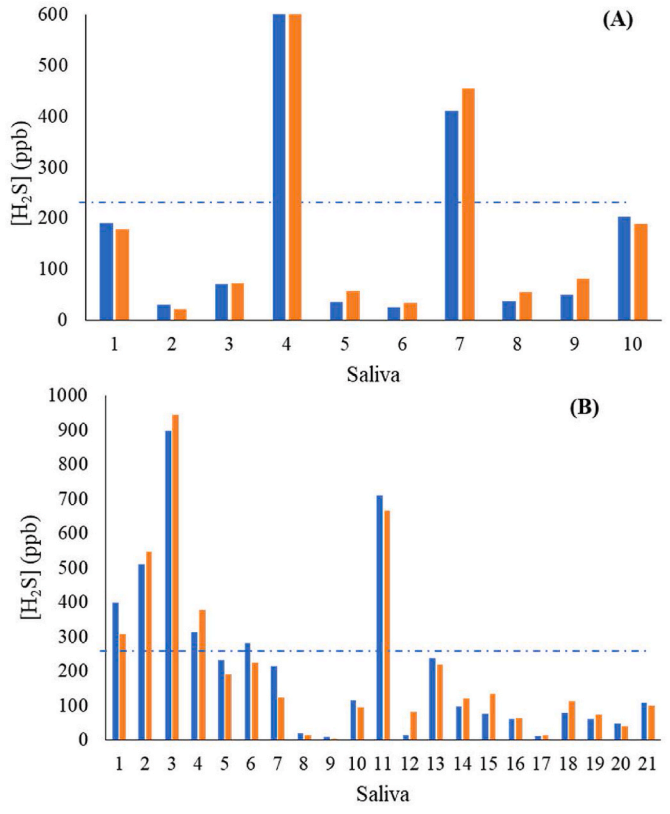

Fig. 6. H_2S μg L^{-1} in saliva real samples at 480 nm and 500 nm. A) Unfiltered, B) Filtered. Microplate format.

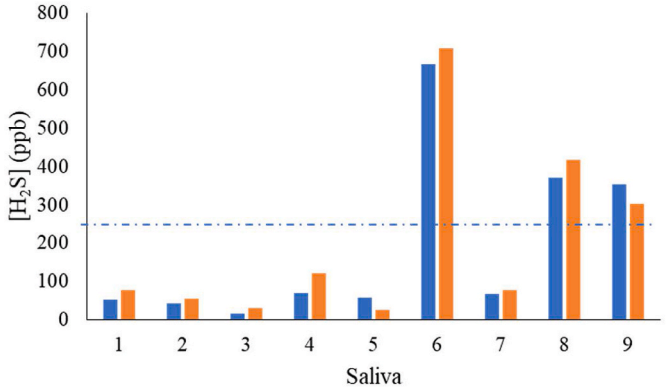

Fig. 7. H_2S μg L^{-1} in saliva real samples at 480 nm and 500 nm. Samples from 1 to 6 the reaction time was 15 min. From 7 to 9 the reaction time was 30 min. Bag format.

production of H_2S, the response time was too short to increase the concentration.

Regarding the amount of H_2S in the samples, most values are lower than 200 μg L^{-1} in the case of filtered (Fig. 6A) and unfiltered saliva (Fig. 6B), which goes into than expected compared to literature values obtained for healthy subjects. Furthermore, several of them are below the experimental detection limit at 500 (70 μg L^{-1}) and 480 nm (30 μg L^{-1}). Samples with H_2S values higher than 300 μg L^{-1} could be explained by problems in the oral cavity such as gingivitis or periodontitis for instance.

In bags, 10 unfiltered saliva samples (non-fortified and fortified) were analyzed at 15 (Samples 1 to 7) and 30 (Samples 8 to 10) minutes. Recoveries are shown at Table 4. Focusing on these results, it can be concluded that there are a wide variety of values since there are samples with very low recoveries (9%, 11%) working at 15 min, which indicates the presence of a considerable matrix effect with a response much lower than expected, others in which recoveries are as expected (80–120%), and a third group with a response greater than it should be (164, 144%). Comparing the values between the two wavelengths studied, recoveries do not differ significantly from each other for the same response time. Working at 30 min, recoveries are very similar, with a mean value of 63 ± 7% (n = 4). This fact indicates that, when calculating the concentration of H_2S in saliva samples, recoveries must be considered. In this case more reproducible results were obtained by using 30 min as reaction time.

Sulfide values expressed as H_2S obtained in each saliva are represented in Fig. 7. Analyzing these results, it can be observed that the concentrations present in saliva samples are similar using the two wavelengths. H_2S concentration ranges found were between 50 and 150 μg L^{-1} in most samples, which correspond to the expected values for healthy subjects. However, in the case of saliva 6, 8 and 9 a higher H_2S value than expected was obtained.

3.4. Microplate format vs bag format

A comparison between the two formats assayed have been performed. Good correlations were achieved by using both formats. Both formats are selective to H_2S (g) and no invasive to the sample. Slightly lower LODs and LOQs were obtained by using microplate format. The sensitivities reached were comparable. In both formats a matrix effect was obtained and the recovery with fortified sample should be employed in order to calculate the concentration in the sample. Concerning to the precision RSD% lower values were obtained by using microplate. One of the main advantages of using microplate format is that several samples (96) can be processed simultaneously. To carry out this assay a Nylon sheet with 96 spots of AgNPs can be used. About to the sample filtration, no improvement in the recovery was observed. Concerning to the time

Table 5
Figures of merit obtained by using RGB coordinates.

Coordinate	a ± s_a	b ± s_b	R^2	LOD (mg/L)	LOQ (mg/L)
R	0.0027 ± 0.0026	0.025 ± 0.004	0.940	0.3	0.9
G	0.007 ± 0.006	0.042 ± 0.011	0.869	0.5	1.4
B	0.008 ± 0.007	0.109 ± 0.011	0.977	0.19	0.6

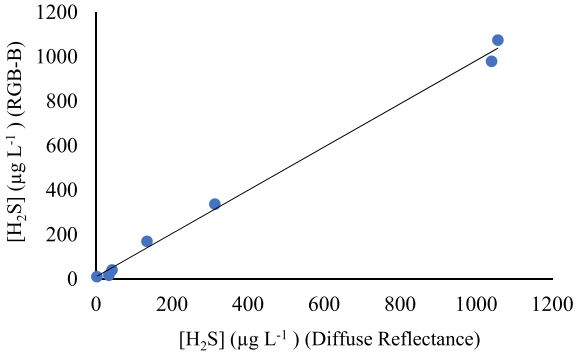

Fig. 8. Lineal regression between H_2S µgL^{-1} in saliva real samples by using conventional diffuse reflectance vs RGB coordinates (B coordinate).

response, working with bag format 30 min were recommended, while 15 or 30 min by using microplate format.

3.5. Comparison between diffuse reflectance and RGB

In order to perform an in-situ analysis, the advantages of the color images from a smartphone were evaluated. The RGB coordinates were obtained. These values were transformed in absorbance applying the expression A = -log (I/I_o) being the I the value of one of the coordinates and I_o the blank value. As can be seen in Table 5, a good correlation was obtained for B coordinate, and a higher sensibility respected the others coordinates. For a certain number of saliva samples, the image measurement was carried out to obtain the corresponding RGB coordinates as a comparison between the two quantification methods proposed in this work. Fig. 8 shows the concentrations obtained by diffuse reflectance and RGB for seven samples, using B coordinate as turned out to be the most sensitive one. Analyzing the results, we can see how the concentrations calculated by the two methods are similar in all the saliva samples analyzed and a good correlation between the results of both methods is obtained. The slope obtained was statistically equal to 1 (0.097 ± 0.028) and the ordinate was equal to zero (10 ± 16) which mean that not statistically differences were between the concentration provided by both methods. According to these results the RGB values can be used as analytical signal, however slightly lower sensitivity and higher LOD and LQD were achieved.

4. Conclusions

In this work it has been confirmed that the bag format and the well microplate format are appropriated for the determination of H_2S in saliva samples using a Nylon sensor with AgNPs retained in a reasonable analysis time (15 min). In addition, LODs and LOQs are satisfactory, obtaining values of 0.019 mg L^{-1} for the LOD and 0.06 mg L^{-1} for the LOQ using the bags format. In the case of the well microplate, values of 0.03 mg L^{-1} for the LOD and 0.09 mg L^{-1} for the LOQ were obtained. When analyzing real saliva samples, a wide recovery range has been obtained for both formats, which indicate the presence of a matrix effect on saliva samples. These recoveries will be used to calculate the H_2S concentration in the sample. Regarding the values of sulfides obtained using bags and microplate, most values were between 20 and 300 µg L^{-1}, as it was expected, although there were several samples in which the amount of H_2S was higher. This determination can be carried out in a laboratory by using conventional laboratory instrumentation or in situ by using the RGB coordinates as analytical signals obtained by processing the images taken by smartphone. The proposed approach provides high sensitivity, selectivity, rapidity, is not invasive and it can be used as indirect method to measure malodor caused by H_2S.

Author contributions

P.Campins-Falcó: Conceptualization, Methodology, Validation, Supervision, writing—review, Funding acquisition; C.Molins-Legua: Conceptualization, Methodology, Validation, Supervision, writing, editing; I. Carrero-Ferrer: Investigation, Validation, Writing – original draft preparation; All authors have read and agreed to the published version of the manuscript

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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