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Combining PDMS Composite and Plasmonic Solid Chemosensors: Dual Determination of Ammonium and Hydrogen Sulfide as Biomarkers in a Saliva Single Test

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Abstract: In recent years, in the field of bioanalysis, the use of saliva as a biological fluid for the determination of biomarkers has been proposed. Saliva analysis stands out for its simplicity and non-invasive sampling. This paper proposes a method for the dual determination of ammonium and hydrogen sulfur in saliva using two colorimetric chemosensors. The ammonia reacts with 1,2-Naftoquinone 4 sulphonic acid (NQS) entrapped in polydimethylsiloxane (PDMS) and the hydrogen sulfide with AgNPs retained in a nylon membrane. The color changed from orange to brown in the case of ammonia chemosensors and from yellow to brown in the H_2S. The experimental conditions to be tested have been established. Both analytes have been determined from their gaseous form; these are ammonia from ammonium and hydrogen sulfur from hydrogen sulfur. Good figures of merit have been obtained by using both measuring strategies (reflectance diffuse and digitalized images). The acquired results show that both sensors can be used and provide good selectivity and sensitivity for the determination of these biomarkers in saliva. Both measurement strategies have provided satisfactory results for the real saliva samples (n = 15). Recoveries on spiked samples were between 70% and 100%. This methodology can lead to possible in situ diagnosis and monitoring of certain diseases and pathologies related with NH_4^+ and/or H_2S, in a fast, simple, cheap and non-invasive way.

Keywords: colorimetric chemosensors; saliva; ammonium; hydrogen sulfide; diffuse reflectance; RGB coordinates; smartphone

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1. Introduction

Saliva is an aqueous fluid found in the oral cavity that plays a vital role in preserving and maintaining oral health [1]. Approximately 93% of the saliva's volume originates from major salivary glands, while the remaining 7% is produced by minor glands [2]. While saliva is sterile when released from the salivary glands, it loses its sterility upon coming into contact with crevicular fluid, food residues, microorganisms, and other substances present in the oral cavity [3]. Saliva typically has a pH ranging from 6.5 to 7.5 and is primarily composed of water (about 99%), along with a smaller concentration of inorganic and organic compounds (about 1%) [4]. Inorganic species mainly include ions like Na^+, K^+, Cl^-, Ca^{2+}, HPO_3^{2-}, HCO_3^-, mg^{2+}, and NH_4^+. Organic components consist of secretion products (urea, uric acid, and creatinine) [5–7], putrefaction products (putrescine and cadaverine) [8], carbohydrates (glucose), amino acids [9], lipids (cholesterol and fatty acids) [10], hormones [11], and over 400 types of proteins [12]. Among the proteins found in saliva are those originating from salivary glands (α-amylase, histatins, cystatins, lactoferrins, lysozymes, mucins, etc.) as well as proteins derived from the bloodstream (albumin, s-IgA, transferrin, etc.) [13,14].

Several intra and extracellular pathways allow saliva to contain substances. This raises the possibility of using saliva for diagnosing certain pathologies [15,16]. The con-

centration of some substances present in saliva varies when a disease affects the body. These substances are called biomarkers and can be used as indicators of a person's health status. The non-invasive sampling method is one of the main reasons for using saliva as a diagnostic fluid; other advantages are easy and non-expensive collection, availability and easy transport and storage. Additionally, the possibilities of interference are minimal since the protein content in saliva is lower compared to blood or serum. The composition of saliva is not as complex and variable as serum. Saliva sampling can be a good alternative to blood/serum, as it is simple, less costly, and safe.

Ammonia (NH_3) plays a significant role in the human body and is considered an important biomarker. It is found in all body fluids, mainly as the ammonium ion (NH_4^+). Concerning saliva samples, ammonium increases saliva pH, which helps neutralize acids and prevent cavities. High levels of ammonia in saliva and breath have been attributed to various kidney [17,18], liver [19], and stomach [20] diseases, making its determination potentially useful for diagnosis. Table 1 compiles some recently published methods based on the use of chemosensors and the analytical parameters obtained for ammonium determination in saliva. Thepchuay et al. [21] developed a paper chemosensor impregnated with bromothymol blue indicator and analyzed saliva samples (n = 10) from healthy individuals. The detected ammonia concentrations ranged from 20 to 90 mg/L. Liu et al. [22] fabricated a sensor consisting of a soap film connected to a conductance detector. The sensor was tested on various matrices, including two saliva samples. The analytical performance parameters were demonstrated, including a linear range of 0–500 µM, a relative standard deviation of 3.2% (n = 10), and a limit of detection of 14 µM (0.2 mg N/L). This method showed a 90–110% recovery rate for saliva.

Table 1. Methods based on sensor for NH_4^+ determination in saliva.

Methods	Lineal Interval (mg/L)	LOD (mg/L)	Reference
Micro-PAD card for measuring total ammonia in saliva	11–50	3	[21]
Soap film as a rapidly renewable and low-cost sensor for detecting ammonia in water and saliva	0.7–500	0.2	[22]
Dissolved ammonia sensing in complex mixtures using metalloporphyrin-based optoelectronic sensor and spectroscopic detection	1.3–17	0.4	[23]
Au-decorated electrochemically synthesised polyaniline-based sensory platform for amperometric detection of aqueous ammonia in biological fluids	0.07–870	0.02	[24]
A paper-based device for the colorimetric determination of ammonia and carbon dioxide using thiomalic acid and maltol functionalized silver nanoparticles	0.6–1700	0.3	[25]
Passive Solid Chemosensor as Saliva Point of Need Analysis for Ammonium Determination by Using a Smartphone	100–700	30	[26]

Typical NH_3 concentrations in gastric juice can vary from ~50 ppm for healthy individuals to ~200 ppm for those infected with H. pylori. Zilberman et al. [23] synthesized a composite based on zinc metalloporphyrins and demonstrated its efficacy on saliva samples, finding concentrations of approximately 26 mg/L. Breath analysis is an alternative, but ammonia presents in the breath at much lower concentrations of 100 ppb–2 ppm as a part of a complex mixture of other volatiles, making NH_3 detection quite challenging. Salivary NH3 concentrations are just slightly lower than those in the gastric juice, starting from ~20 ppm.

Korent et al. [24] prepared an electrode composed of a polyaniline polymer and gold nanoparticles but only tested its efficacy on artificial saliva. Finally, Sheini [25] developed a paper chemosensor with functionalized silver nanoparticles and conducted a study on concentration levels in healthy individuals and patients with kidney problems. In healthy individuals, values ranged between 120 and 400 mg/L, while in patients with kidney problems, values exceeded 500 mg/L. Monforte et al. [26] developed a NQS polymeric chemosensor for ammonium in saliva samples. The lineal range was between 100 and 700 mg/L and the limit of detection was 30 mg/L.

Regarding hydrogen sulfide (H_2S), this is traditionally known for being a toxic gas with a rotten egg smell; it serves as a mediator in many biological systems [27]. Various studies have revealed that H_2S participates in the regulation of several physiological and pathological conditions in mammalian systems [28]. In the human body, an increase in H_2S concentration is associated with respiratory conditions such as chronic bronchitis, emphysema, pneumonia, or cardiovascular-related diseases, such as hypertension [29]. In the oral cavity, H_2S appears as a bacterial waste product, and it plays a crucial role in the bacterial-induced inflammatory response in oral diseases, such as gingivitis and periodontitis [30]. The accumulation of H_2S, among others, is one of the contributors to the development of halitosis or bad breath [31]. Additionally, it has been demonstrated that volatile sulfur compounds (VSCs), especially H_2S, induce the apoptotic process in various types of cells within oral structures [32]. For these reasons, the determination of H_2S in saliva or breath could be utilized for diagnosing or monitoring the progression of oral diseases such as periodontitis.

Table 2 compiles some recently published methods based on the use of chemosensors and the analytical parameters for H_2S determination in saliva. Zaorska et al. [33] have developed a fluorescent probe for salivary H_2S concentration in healthy volunteers. The concentrations were within a range of 1.641–7.124 μM. Kroll et al. [34] also synthesized fluorescent probes for saliva concentration. The values found ranged from 0.055 to 0.3 mg/L. Ahn et al. [35] developed a paper chemosensor impregnated with silver nitrate to detect H_2S produced by various bacteria. Cha et al. [36] created a colorimetric chemosensor based on lead acetate nanofibers as a possible halitosis diagnostic. Samples from healthy individuals (n = 10) were analyzed, yielding results below the detection limit (0.2 mg/L). Finally, Carrero-Ferrer et al. [37] used a plasmonic chemosensor based on silver nanoparticles to analyze saliva samples (n = 10), most of which presented concentrations below 0.2 mg/L.

Table 2. Methods based on sensors for H_2S determination in saliva.

Article	Lineal Interval (mg/L)	LOD (mg/L)	Reference
Salivary Hydrogen Sulfide Measured with a New Highly Sensitive Self-Immolative Coumarin-Based Fluorescent Probe	0.05–0.56	0.02	[33]
Sensitivity of salivary hydrogen sulfide to psychological stress and its association with exhaled nitric oxide and affect	0.08–0.56	0.02	[34]
Simple and Sensitive Detection of Bacterial Hydrogen Sulfide Production Using a Paper-Based Colorimetric Assay	0.02–2.8	0.008	[35]
Sub-Parts-per-Million Hydrogen Sulfide Colorimetric Sensor: Lead Acetate Anchored Nanofibers toward Halitosis Diagnosis	0.6–5	0.2	[36]
Plasmonic sensor for hydrogen sulphide in saliva: Multisensor platform and bag format	0.06–1	0.02	[37]

In this paper, a dual determination of NH_4^+ and H_2S have been proposed in saliva samples using two patented chemosensors from the minTOTA group [38,39]. The chemosensor for ammonium is based on a composite of PDMS, tetraethyl orthosilicate (TEOS), Silica NPs, ionic liquid (IL) and 1,2-naphthoquinone-4-sulfonate (NQS) as a chromophore [26,38]. The amonia will react with the NQS entrapped in the PDMS. The IL we used was1-methyl-3-octylimidazolium hexafluorophosphate.

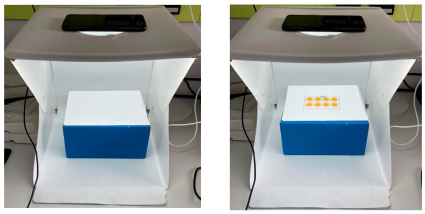
The chemosensor for H_2S is a plasmonic sensor based on AgNPs retained on nylon [37,40]. The H_2S will interact with the AgNPs affecting the plasmonic band. The experimental conditions to be determined have been established in order to determine both compounds in a single test. The selectivity of the chemosensor and the interference between the analytes have been studied. Two different methodologies to measure the analytical responses have been used and compared: the reflectance diffuse and the RGB color coordinate obtained by using a smartphone. The application to real saliva samples has been realized, and no matrix effect has been observed. The use of a smartphone and the RGB coordinates is an alternative to conventional spectral instruments.

2. Materials and Methods

The dispersion of silver nanoparticles of 20 nm (0.02 mg/mL in an aqueous buffer containing sodium citrate as a stabilizer), glycerol (≥99%), sodium bicarbonate, silica nanoparticles, 1-methyl-3-octylimidazolium hexafluorophosphate, 1,2-naphthoquinone-4-sulfonate, and tetraethyl orthosilicate, were obtained from Sigma-Aldrich (Germany). The silicone elastomer base (PDMS) Sylgard® 184 and the curing agent Sylgard® 184 were provided by Dow Corning (MI, USA). Ammonium chloride was obtained from Probus S.A. (Barcelona, Spain). Water (ultrapure quality) and 85% phosphoric acid were provided by Panreac (Spain). Sodium sulfide and sodium carbonate were obtained from Scharlau (Australia) and VWR Chemicals (USA), respectively.

The nylon membranes (pore size 0.22 µm) were obtained from Filter-Lab (USA). The air sampling bags (5 × 7 cm) were purchased from Aliexpress.

The white box with LED lighting (20 × 20 × 20 cm) PULUZ was obtained from Amazon (Figure 1). The Hamilton 1750 syringe (500 µL) was provided by Fisher Scientific. The vacuum pump was obtained from KNF (Germany).



(**A**) (**B**)

Figure 1. (**A**) Device used to obtain the images without chemosensors. (**B**) With chemosensors.

The ultrasonic cleaner (LBX Instruments), magnetic stirrer (Ecostir, DLAB, Spain) and drying oven (SLW 115, POL-EKO) were used in the synthesis of the NH_3 chemosensors. Ultrapure water used in the preparation of solutions and synthesis of the H_2S chemosensor was obtained using a water purifier (Nanopure, Adrona). An 8-well plate Labox (Barcelona, Spain) 95 × 57 dimensions, 15 mm diameter, made of polystyrene was used. Saliva samples were centrifuged using a centrifuge for Eppendorf tubes (MC15K series, LBX Instruments). The pH of the solutions and samples was measured using a benchtop pH meter (pH50+ DHS, (Xylem Analytics, Germany) with a pH microelectrode (METRIA). For obtaining UV-Vis spectra of the chemosensors, a UV-Vis spectrophotometer (Varian Cary 60, Agilent, USA) equipped with a diffuse reflectance probe (Harric Scientific Products, New York USA) was used. Photographs of the chemosensors were taken with a smartphone (Xiaomi Redmi Note 11S) using the "Pro" mode of the camera, ISO: 2000. The images were taken

using a smartphone and a white box with 60 (30 × 2) LED light (Figure 1). RGB coordinate decomposition was performed using GIMP software (Version 2.10.34).

3. Procedures

3.1. NH_3 Chemosensor

Weigh NQS (0.4%) and IL (7.8%) and stir for 15 min until a homologue is obtained. Then, weigh PDMS (35%) in the same beaker and continue stirring for 5 min until it is completely homogeneous. In a separate beaker, weigh silica (0.8%) and TEOS (56%), and introduce them into an ultrasonic bath for about 20 s. The membrane was doped with 1-methyl-3-octylimidazolium hexafluorophosphate in order to increase the permeability of the membrane. Pour the formed dispersion onto the beaker containing the mixture of NQS, IL, and PDMS and let it stir for 4–5 h until the mixture is completely homogeneous. Once the mixture is homogeneous, add the curing agent (3.5%) and stir for 1 min to incorporate it into the mixture. Finally, weigh 0.2 g of the mixture into each well of an 8-well plate and let them rest in an oven at 40 °C for 24 h to gel. From each chemosensor, 4 smaller chemosensors can be obtained using a paper punch. Store the chemosensors in parafilm and cover them with aluminum foil at −15 °C until use.

3.2. H_2S Chemosensor

Rectangle of proportions 8 × 13 cm (size of a 96-well microplate) is drawn on the nylon membrane (0.22 µm) and cut out. The assembly used consists of a rectangular acrylic box with a hollow 96-well microplate on the top. Additionally, the box has an outlet on one side where the vacuum pump is connected. The cut nylon membrane is placed on top of this box, and another hollow 96-well microplate is placed on top of the membrane. Then, using a multichannel micropipette, 110 µL of ultrapure water is added to each well to moisten the membrane. Finally, 110 µL of the silver nanoparticle dispersion is added and a vacuum is applied for 10 s. The chemosensors are moistened with ultrapure water, covered with parafilm and aluminum foil, and stored in darkness at 4 °C until use. After use (exposure to H_2S), glycerol was used to cover the AgNPs spot. Finally, the response was registered. As well as the chemosensor of ammonium, absorbance from the reflectance diffuses and the color coordinates from the digital images were used as analytical responses. Figure 1 displays images of the smartphone equipment. The analytical signal (Absorbance) was normalized according to Carrero et al. [37]

3.3. Determination of Ammonia and Hydrogen Sulfur

For the determination of the analytes, first, the bottom part of the sampling bag is cut off, and both chemosensors, attached with tape, along with a stir bar, are inserted. The chemosensors consist of NQS entrapped in PDMS and AgNPs retained in a nylon membrane. The bag is then sealed with heat. Next, using a Hamilton syringe, 200 µL of the standard (mixture of H_2S and NH_4^+) or sample solutions are introduced, and 20 µL of a 0.85% phosphoric acid solution is added to adjust the pH to approximately 4–5, promoting the formation of H_2S. Subsequently, 30 mL of clean air is added using a syringe, and the mixture is stirred for 10 min. During this time the interaction between H_2S and AgNPs-nylon takes place. After this time, 100 µL of a 2M CO_3^{2-}/HCO_3^- buffer (pH = 11.5) is added to promote the formation of NH_3, and the mixture is stirred for 15 min. During this time, the reaction of NH_3 and NQS-PDMS takes place (Figure 2). Once completed, the chemosensors are removed from the bag, one drop of glycerol is added to the H_2S chemosensor, and they are either measured using diffuse reflectance or photographed to obtain their RGB values. The chemosensors can be stored in darkness at 4 °C until the signals are registered (Figure 1). For the H_2S chemosensor, the normalized spectrum was obtained [37].

Multicomponent standard solutions of ammonium and hydrogen sulfide were prepared using ammonium chloride and sodium sulfide, with concentration intervals of 0.05–2 mg/mL and 0.1–1.5 mg/L, respectively. For sample fortification, 20 µL of a multi-

component solution containing 4 mg/mL of ammonium and 5 mg/L of hydrogen sulfide were added. Samples with NH_4^+ or H_2S concentrations above the linear range were diluted by adding 100 µL of sample and 100 µL of water to the vial.

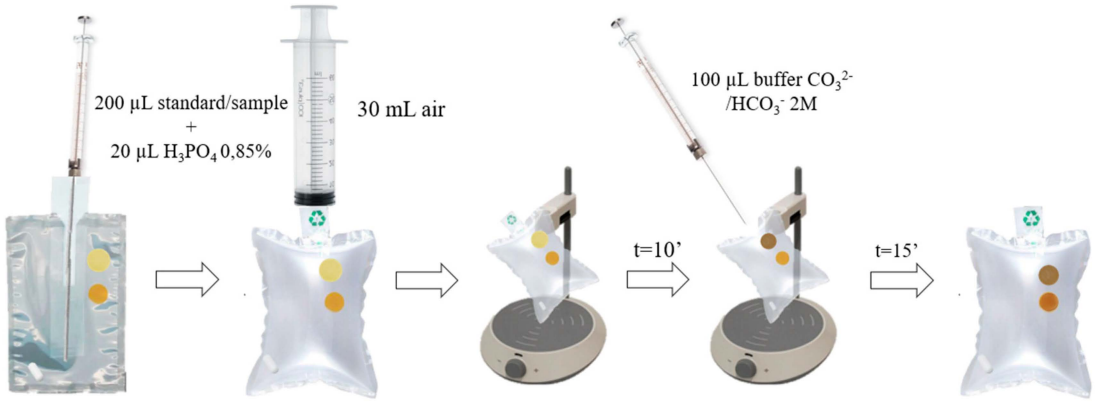


Figure 2. Schedule of the procedure employing both chemosensors. H_2S in acidic medium followed by 10 min reaction time. Basic medium followed by 15 min reaction time between NH_3 and the NQS-PDMS chemosensors.

3.4. Real Saliva Samples

Sample Collection and Treatment

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. Volumes between 0.5 and 1 mL were taken. Fifteen saliva samples were collected within a wide age range (20–60 years). Eight were female and seven were male. The samples were taken at room temperature and before meals, as the composition of saliva can vary after eating. The samples were centrifuged at 3500 rpm for 10 min. All analyses were performed within 4 h of sample collection. Prior to analysis, samples were centrifuged at 10,000 rpm for 10 min. For spiked saliva samples, a suitable volume of sulfide solution was added.

4. Results and Discussion

4.1. Selection of the Experimental Conditions

According to previous publications [38–40], the optimal measurement wavelengths were established at 480 nm and 560 nm for H_2S and NH_4^+, respectively (Figure 3). Measuring at these wavelengths provides a higher sensitivity. The wavelengths were selected based on intensity. Concerning the measurement of RGB color coordinates for both analytes, the three coordinates were considered. In Figure 4 are shown the calibration graphs for H_2S and NH_3 using the three coordinates (R, G and B coordinates). As can be seen, the G coordinates provided the best linearity and sensitivity. According to these results, the G coordinate was selected for further experiments. Similar results were obtained for both analytes.

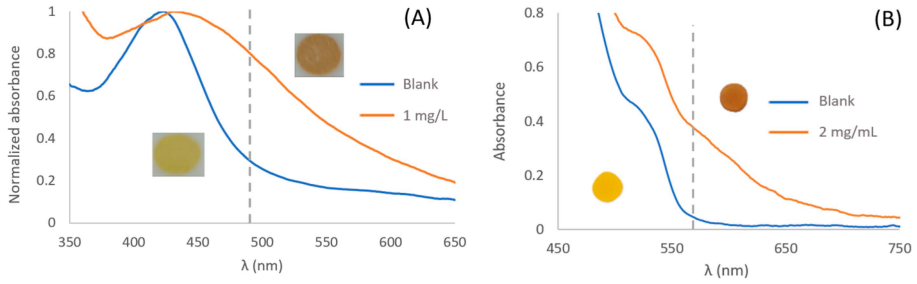


Figure 3. UV-Vis spectra for H_2S chemosensor (**A**) and (**B**) NH_3 before and after analyte chemosensor exposure during 15 min. The dashed line represents the measured wavelength.

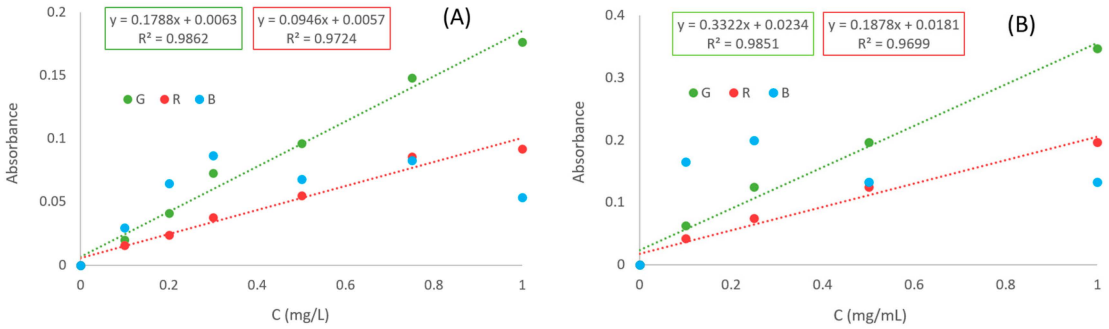


Figure 4. Calibration graphs of H_2S (**A**) and NH_3 (**B**) with different RGB components.

The RGB coordinates are transformed in absorbance using the Lambert–Beer equation:

$$A = -\log_{10}\left(\frac{I}{I_0}\right) \quad (1)$$

where I is the RGB coordinates of the standard and I_0 is the blank value. This equation has been taken from the literature [37].

The amount of analyte volatized after the chemical treatment was evaluated. Concerning the H_2S determination, the slope at 10 min and 15 min are statistically equal. This means that the H_2S has been released from the solution in 10 min. This was shown by Jornet et al. [40]. The remaining solution contained less than 1% of the standard content. These experiments were performed using the methylene blue method. These times were sufficient to establish a correlation between the gas releases in the solution and the original substance concentration in the solution.

In relation to NH_4^+, when the remaining solution was analyzed by using the Berthelot method, the amount released from the solution was nearly 100%. In Table 1, the absorbance of the remaining solution after 15 min of reaction is shown. The amount remaining was less than the LODs and the % volatilized was <99%.

One important parameter to study is the pH of the standard solutions and samples, as the analytes diffuse to the chemosensor in a gaseous form. Both the prepared standard solutions and saliva samples have a pH value of approximately 7. At this pH, the predominant species are H_2S, HS^-, and NH_4^+, considering the acidity constants (pKa_1 = 7 and pKa_2 = 12.9 for hydrogen sulfide, and pKa = 9.3 for ammonia). Under these pH conditions, ammonia is present as NH_4^+ in the solution and does not dissociate as NH_3. Therefore, to determine ammonium, it is necessary to raise the pH of the solutions and samples above 9 to convert it into NH_3 and allow it to be released. To shift the equilibrium towards the formation of NH_3, 100 µL of a 2M CO_3^{2-}/HCO_3^- buffer (pH = 11.5) was added, which raised the pH to 10.6–11.5 depending on the saliva sample. On the other hand, at pH 7, part of the H_2S is protonated and is released from the solution, reaching the chemosensor. To shift the equilibrium and facilitate the formation of H_2S, 20 µL of 0.85% phosphoric acid was added, causing a decrease in pH to 4–5, depending on the saliva sample. Two cal-

ibrations were prepared, one with the addition of phosphoric acid and one without, to assess its effect. It was observed that the addition of phosphoric acid significantly increases sensitivity by more than 50% (Figure 5). This is because lowering the pH to 5 shifts the acid-base equilibrium, causing the predominant species to be H_2S (g).

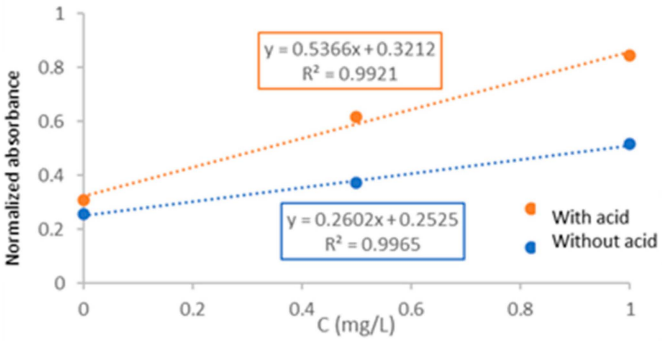


Figure 5. H_2S calibration graph with and without adding phosphoric acid (exposure time 15 min). Data provided using diffuse reflectance.

The effect of the thickness of the chemosensor was evaluated. Two depths were tested (1 and 2 mm). The result obtained indicated that the higher the depth, the higher is the response. The next parameter studied was the exposure time required for the chemosensors to respond to the analyte concentration. As mentioned earlier, the chemosensors respond to analytes in the gaseous state, so they must be released from the solution and diffused to the chemosensors. This parameter is particularly crucial for the ammonia chemosensor because in addition to reaching the chemosensor, ammonia must diffuse through the polymeric matrix and react with the NQS. This limits the signal provided via the chemosensor due to the time it is exposed to the analyte. For the study, two ammonia standards of 0.25 and 0.5 mg/mL were prepared, and the chemosensor was exposed for 15 and 30 min. A better sensitivity was obtained by using 30 min. Similarly, two calibrations (Analytical signal vs. H_2S concentration) were performed at different exposure times (10 and 15 min). It is observed that for exposure times of 10 and 15 min, the results obtained are similar (Figure 6). Both the y-intercept value and the slope of the calibration curve are similar. Thus, 10 min was selected as the exposure time, since shorter times provide lower sensitivity.

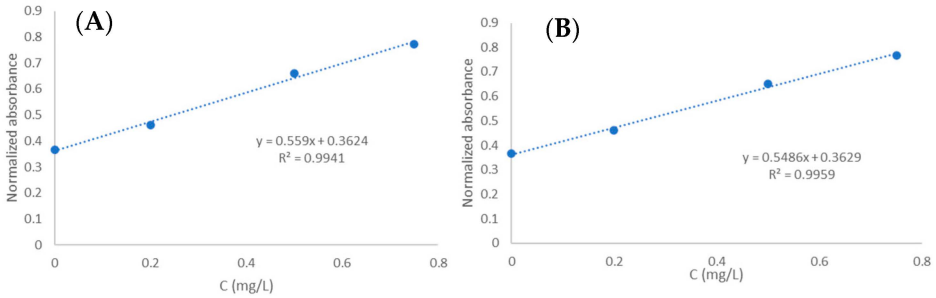


Figure 6. Calibration graph for H_2S for different time of chemosensor exposure (**A**) 10 min, (**B**) 15 min.

4.2. Study of the Chemosensor Response in Presence of the Other Analyte

After selecting the measurement conditions, a study was conducted to investigate the potential interferences of the analytes among themselves. To accomplish this, the AgNPs chemosensor was exposed to a 2 mg/mL ammonium standard, while the NQS chemosensor was exposed to a 1 mg/L hydrogen sulfide standard. As shown in Figure 7A, the AgNPs chemosensor only responds to the presence of hydrogen sulfide, while the NQS chemosensor responds to the presence of ammonia (Figure 7B). Therefore, both analytes can be determined without interference. The possible interference between the analytes when determining them together was studied. The standard solutions of NH_4^+ and H_2S were

prepared, as well as a multicomponent standard solution with the same concentrations. Measurements were taken separately and together, and the results were obtained through diffuse reflectance. The coefficient of variation was calculated for both determinations. An RSD value of 0.9% (n = 3) was obtained for the determination of H_2S, and 3% (n = 3) for NH_4^+.

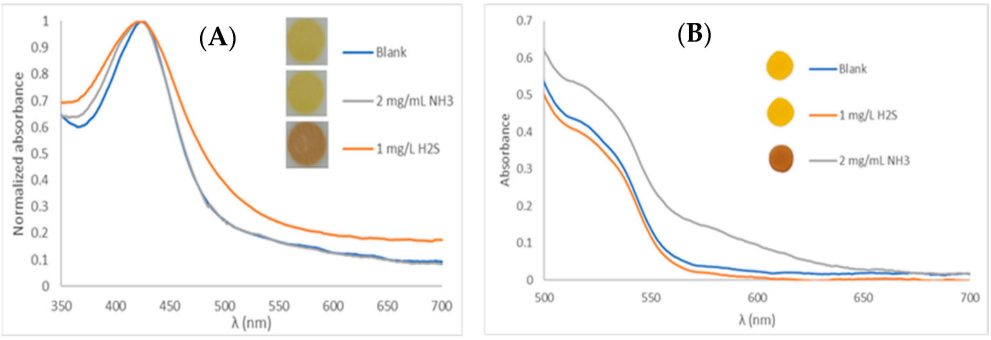


Figure 7. (**A**) Spectra of the H_2S chemosensor exposed to standard solutions of NH_3 and H_2S. (**B**) Spectra of the NH_3 chemosensor exposed to standard solutions of NH_3 and H_2S.

In this method, the determination of H_2S in an acidic medium was performed first by acidifying the sample. After the reaction time, the medium was alkalized (Figure 2). This approach ensures that when raising the pH of the solution/sample and releasing NH_3, the chemosensor signal is not affected by the presence of the other analyte. After the reaction of both chemosensors, H_2S and NH_3, the analytical responses were measured in the same sample.

4.3. Analytical Parameters of H_2S and NH_4^- Determination

Once the experimental conditions were established, the analytical parameters were determined for both compounds. Table 3 displays the detection and quantification limits, as well as the linear range. The calibration graphs are shown in Table 4. The LODs and LOQs were calculated based on 3 and 10 times the standard deviation of the blank divided by the slope of the calibration curve. To establish the linear range for each analyte, standards with concentration ranges of 0.05–2 mg/mL and 0.1–1.5 mg/L were prepared for ammonium and hydrogen sulfide, respectively. In Figure 8A,B are shown the spectra and the colorimetric card. As can be seen, the color changes with the concentration from yellow to brown for the H_2S chemosensor and from yellow-orange to brown for the NH_3 chemosensor. The NQS reacts with ammonia and primary and secondary amines giving different colors. In this case, the color change was associated with ammonia, which was the most abundant compound in saliva samples. Regarding the determination of H_2S, a 10-min exposure time was selected, as shorter times showed a lower response. As can be seen in Table 3, slightly lower LODs were obtained by measuring with diffuse reflectance. As for the NH_3 chemosensor, the sensitivity increased substantially when experiments were conducted with a 30-min exposure time. The best LODs and LOQs were achieved with diffuse reflectance and a 30-min exposure time. However, it should be noted that with longer exposure times, the linear range is reduced.

Table 3. Calibration parameters obtained for both chemosensors and different conditions. [(a)] 10 min exposure time, [(b)] 15 min exposure time, [(c)] 30 min exposure time.

	LOD (mg/L)	LOQ (mg/L)	Lineal Interval
Diffuse Reflectance (H_2S)	0.02 [(a)]	0.06	0.06–1 mg/L
RGB Coordinates (H_2S)	0.03 [(a)]	0.10	0.10–1 mg/L
Diffuse Reflectance ((NH_4^+)	0.02 [(b)]	0.06	0.06–2 mg/mL
	0.007 [(c)]	0.02	0.02–0.5 mg/mL
RGB Coordinates (NH_4^+) [(c)]	0.03 [(b)]	0.10	0.10–1 mg/mL
	0.02 [(c)]	0.06	0.06–0.5 mg/mL

Table 4. Parameters of the calibration graph for both chemosensors and at different conditions. Experiments performed at different exposure times.

	Time	$b \pm s_b$	$a \pm s_a$	R^2
Diffuse reflectance (H_2S)	10 min	0.54 ± 0.01	0.344 ± 0.005	0.998
Coordinates RGB (H_2S)	10 min	0.179 ± 0.009	0.006 ± 0.004	0.990
Diffuse reflectance (NH_4^+)	15 min	0.159 ± 0.004	0.084 ± 0.003	0.997
	30 min	0.474 ± 0.018	0.077 ± 0.004	0.994
Coordinates RGB (NH_4^+)	15 min	0.33 ± 0.02	0.023 ± 0.012	0.990
	30 min	0.57 ± 0.03	0.007 ± 0.006	0.993

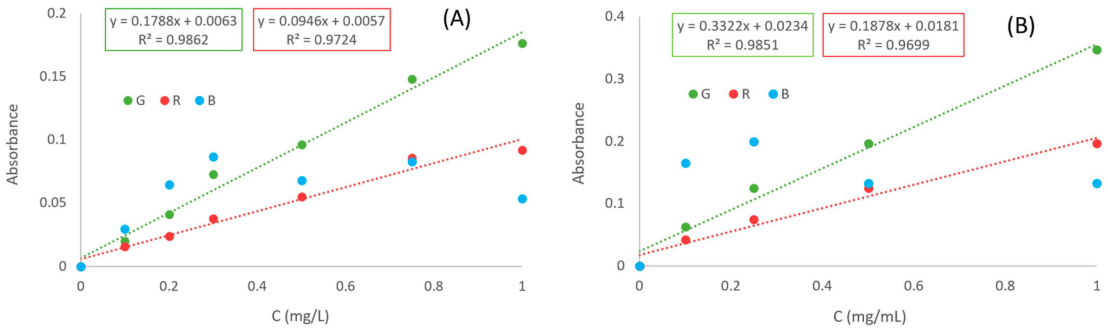


Figure 8. (**A**) Spectra of the H_2S chemosensor exposed to standard solutions of H_2S; colorimetric card at different concentrations of H_2S. (**B**) Spectra of the NH_3 chemosensor exposed to standard solutions of NH_4^+; colorimetric card at different concentrations of NH_4^+.

Table 4 shows the calibration parameters. For H_2S, an exposure time of 10 min was selected. Two exposure times were tested for NH_4^+, 15 and 30 min. The sensitivity was higher with longer exposure time (30 min).

The repeatability was the precision of the method. This was studied by measuring a standard solution of H_2S 0.5 mg/L for the H_2S chemosensor and 0.5 mg/mL of ammonium for the NH_3 chemosensor. The relative standard deviations (RSD%) were calculated for intraday (n = 5) and interday (n = 3) measurements, using exposure times of 10 min for the H_2S sensor and 15 and 30 min for the NH_3 sensor. The intraday precision provided better results than the interday precision.

The obtained results demonstrate that diffuse reflectance provides slightly higher precision compared to using RGB coordinates. Lower values than 2% of RSD intraday were obtained for diffuse reflectance, and values lower than 12% for interday were achieved (Table 5). No significant differences in precision were obtained for both analytes and both measurement procedures. Concerning the exposure times for NH_4^+, 15 and 30 min were assayed. The 30 min showed better precision than shorter times.

Table 5. The intraday and interday precision expressed as RSD% for both chemosensors by using diffuse reflectance and RGB coordinates.

		RSD Intraday (n = 5)	RSD Interday (n = 3)
Diffuse reflectance (H_2S)	10 min	1.3%	9.3%
Coordinates RGB (H_2S)	10 min	3.2%	12.1%
Diffuse reflectance (NH_4^+)	15 min	1.9%	11.5%
	30 min	1.7%	9.7%
Coordinates RGB (NH_4^+)	15 min	2.4%	11.1%
	30 min	2.1%	10.4%

4.4. Application to Real Samples

Fifteen samples taken before meals were analyzed. The analyses were performed within 4 h of sample collection to prevent variations in analyte concentrations due to the potential presence of microorganisms. Prior to analysis, the samples were centrifuged at 3500 rpm for 10 min to separate any food remnants and microorganisms.

The amount of the sample taken was 200 µL. Sample fortification was carried out by adding 20 µL of a multicomponent solution containing concentrations of 0.4 mg/mL for NH_4^+ and 0.5 mg/L H_2S. Samples with concentrations outside the calibration range were diluted by half by adding 100 µL of the sample and 100 µL of ultrapure water.

All the samples were analyzed using diffuse reflectance and decomposition into RGB coordinates. The found concentration in the samples, using both measuring procedures, was calculated and compared by applying a lineal regression (Figure 9). For both analytes, the slope obtained was statistically 1, being the value $b_1 \pm Sb_1$ equal to 0.9711 $\pm$ 0.035 for H_2S and 1.077 $\pm$ 0.0847 for (NH_4^+). The ordinate was equal to zero (0.0396 $\pm$ 0.0223) for H_2S and (0.0104 $\pm$ 0.01469) for NH_4^+. The results obtained indicate that the found concentrations estimated by both procedures are equal statistically. Similar results were obtained by applying the *t* test for paired samples. The tabulated t-values for α = 0.05 and 14 degrees of freedom for NH_4^+ and H_2S, respectively, were 0.999 and 0.841, and when t tabulated = 2.14. Since the experimental t-values are smaller than the tabulated t-values and the *p*-values are higher than 0.05, it can be stated that the results are statistically comparable for both NH_4^+ and H_2S by using both measuring methodologies.

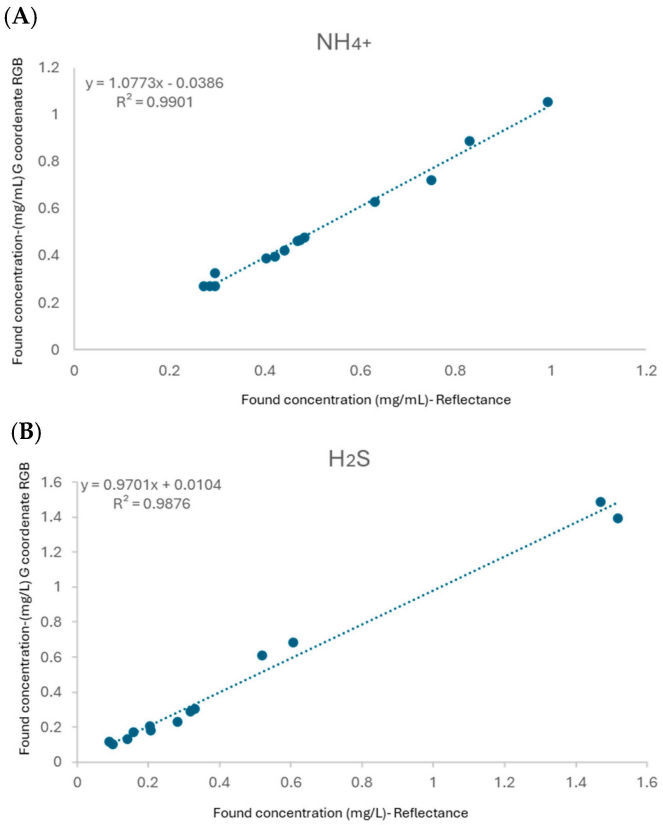


Figure 9. Lineal regression of found concentration of NH_4^+ (**A**) and H_2S (**B**) in the real saliva samples by diffuse reflectance and coordinate G.

In order to evaluate the reliability (accuracy) of the method, fortified samples were processed. All fortified samples were analyzed using diffuse reflectance and decomposition into RGB coordinates. The % recovery for both analytes and both methodologies for measuring was calculated. In Figure 10A are shown the % recoveries for NH_4^+ and Figure 10B shows this for H_2S. A *t* test for paired samples was applied to compare the results obtained with reflectance and coordinate RGB values. The tabulated value of $\alpha = 0.05$ and n = 14 degrees of freedom was 2.14. The values of the *t* test obtained were 2.09 and 0.31 for NH_4^+ and H_2S, respectively. The *t* test was lower than the t tabulated, thus, no significant differences between both the methodologies were obtained.

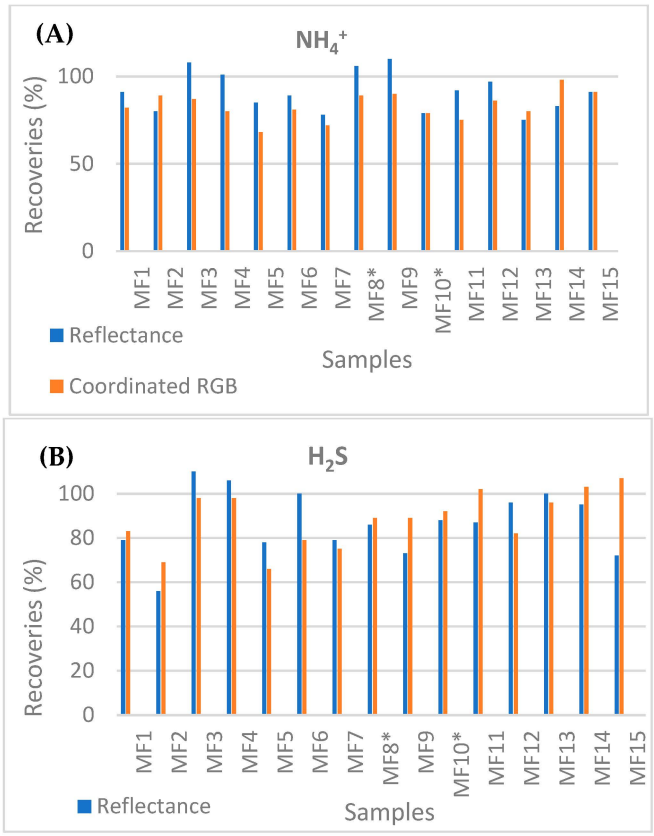


Figure 10. (**A**) Recoveries (%) for fortified samples with NH_4^+. (**B**) Recoveries (%) for fortified samples with H_2S by using reflectance and coordinates RGB. (*) Diluted samples.

The percentage recoveries are shown in Table 6. Both methods yield recoveries between 80 and 102%, and 72 and 96 for ammonium using reflectance diffuse and coordinates RGB, respectively. For H_2S, the recoveries were 72 and 102% and between 76 and 102% for the reflectance diffuse and coordinates RGB, respectively. Table 6 presents the average and standard deviation for these recoveries for each analyte determined via both methods. According to these results, the recoveries obtained for both analytes and both methodologies were between 70 and 100%. With these results, it can be concluded that no significant matrix effect was present in the method.

Table 6. Recoveries (%) in fortified samples with NH_4^+ and H_2S.

		NH_4^+	H_2S
Recovey (%)	**Diffusse reflectance**	91 ± 11	88 ± 15
	Coordinates RGB	84 ± 12	88 ± 13

The selectivity of the method was already performed by Campins et al. [38,39] and Carrero et al. [37,40]. Any interference was observed. The NQS-PDMS chemosensor was selective to ammonia and primary and secondary amines. Due to the amine response being very low, it was considered that the chemosensor response was due to the ammonia. The chemosensor AgNPs was selective with volatile sulfide compounds (VSCs) like H_2S, CH_3SH, and $(CH_3)_2S$. The result can be expressed as H_2S content, due to the response of the other compounds being very low. Compounds such as ethanol, acetone and ammonia were tested. It has been reported that these compounds can be found at high concentrations in breath and are related to several diseases. Ethanol at a higher concentration than 20 ppbv is indicative of diabetes and hyperglycaemia, acetone between 300 and 500 ppbv is associated with lung cancer and 1000–4000 ppbv of ammonia is related to renal failure.

Also, methanol, propanol, formaldehyde and toluene were associated with lung cancer at concentrations > 100 ppbv and 10 ppbv for toluene. The membranes were exposed to these compounds at 5000 ppbv and any change in color or shift of the plasmon band was observed.

Most of saliva samples have ammonium concentrations below 0.5 mg/mL and hydrogen sulfide concentrations below 0.3 mg/L. The sensors were sensitive enough to detect biomarkers for disease [21,37]. Korent et al. [24] reported the amount of ammonium in patients with end-stage renal disease undergoing hemodialysis (HD) gave values of ammonium before HD between 0.13 and 0.72 mg NH_4^+/mL. These values can be observed using minTOTA's sensor (0.100 to 0.700 mg/mL).

Concerning the H_2S sensor, Kroll et al. [34] also synthesized fluorescent probes for saliva concentration. The values found ranged from 0.055 to 0.3 mg/L. The concentration observed via minTOTA's group was from 0.06 to 1 mg/L, with LODs of 0.02 mg/L. Zaorska et al. [33] determined ammonia and hydrogen sulfide. These values fall within the range of 0.05 to 0.5 mg/mL for ammonium and 0.05 to 0.3 mg/L for hydrogen sulfide.

These results are consistent with values reported for healthy individuals by Monforte Gomez [26] for NH_4^+ and Carrero et al. [37] for H_2S. However, some of the analyzed samples exceed these values, which could be attributed to the certain diseases mentioned that are linked to renal, hepatic, gastric or oral diseases [41,42].

5. Conclusions

In the present work, it has been demonstrated that NH_4^+ and H_2S can be simultaneously determined by using colorimetric chemosensors. Two patented chemosensors have been used, one based on a PDMS composite for ammonium and another with AgNPs retained on a nylon support for H_2S. In this work, both determinations have been performed on the same sample. The sample pH is a determinant parameter. The pH should be stablished depending on the pKa of the analyte. Acid conditions are required to determine H_2S while basic pH is demanded for NH_3. The optimal conditions have been established in order to obtain a selective and sensitive procedure. The reaction time and the study of interference have been performed. For H_2S, no differences in the response (10 and 15 min) were observed. For NH_3, 30 min provided better results.

Analytical signals have been obtained by using diffuse reflectance and coordinate RGB. Slightly better results were obtained using diffuse reflectance. However, it has been proved that the RGB measurements are a good alternative to diffuse reflectance in the lab. This methodology is inexpensive, fast, easy to use and can be used for in situ analysis. Besides this, the assay presented good accuracy, precision and sensitivity. The study of the standard addition and the recovery factor indicate that there is not a matrix effect and the % recovery is nearly 100%. Considering all this, it can be concluded that a satisfactory determination of NH_4^+ and H_2S in saliva can be carried out by using this non-invasive method with the chemosensor. The found concentrations in the samples agree with those found in the literature. The cost of the chemosensor and the instrumentation (e.g., smartphone) employed made this a very low-cost procedure.

6. Patents

P. Campíns-Falcó, Y. Moliner-Martínez, R. Herráez Hernández, C. Molins-Legua, J. Verdú-Andrés, N. Jornet-Martínez, Passive Sensor for In-Situ Detection of Amines in Atmospheres, ES2519891B1, 2013.

N. Jornet-Martínez, A.I. Argente-García, P. Campíns-Falcó, C. Molins-Legua, Y. Moliner-Martínez, R. Herráez-Hernández, J. Verdú-Andrés, Colorimetric Sensor Based on Silver Nanoparticles for the Determination of Volatile Sulfur Compounds, EP3467476, 2019.

Author Contributions: P.C.-F.: Conceptualization, methodology, validation, supervision, writing—review, funding acquisition; C.M.-L.: Conceptualization, methodology, validation, supervision, writing, editing. S.M.-C.: methodology, investigation, validation. B.M.-G.: methodology, investigation, validation. All authors have read and agreed to the published version of the manuscript.

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References

1. Milanowski, M.; Pomastowski, P.; Ligor, T.; Buszewski, B. Saliva–Volatile Biomarkers and Profiles. *Crit. Rev. Anal. Chem.* **2017**, *47*, 251–266. [CrossRef] [PubMed]
2. Llena-Puy, C. The Rôle of Saliva in Maintaining Oral Health and as an Aid to Diagnosis. *Med. Oral Patol. Oral Cir. Bucal.* **2006**, *11*, 449–455.
3. Tenovuo, J. Salivary Parameters of Relevance for Assessing Caries Activity in Individuals and Populations. *Community Dent. Oral Epidemiol.* **1997**, *25*, 82–86. [CrossRef]
4. Mikkonen, J.J.W.; Singh, S.P.; Herrala, M.; Lappalainen, R.; Myllymaa, S.; Kullaa, A.M. Salivary Metabolomics in the Diagnosis of Oral Cancer and Periodontal Diseases. *J. Periodontal. Res.* **2016**, *51*, 431–437. [CrossRef]
5. Guan, Y.; Chu, Q.; Ye, J. Determination of Uric Acid in Human Saliva by Capillary Electrophoresis with Electrochemical Detection: Potential Application in Fast Diagnosis of Gout. *Anal. Bioanal. Chem.* **2004**, *380*, 913–917. [CrossRef]
6. Nagler, R.M.; Hershkovich, O.; Lischinsky, S.; Diamond, E.; Reznick, A.Z. Saliva Analysis in the Clinical Setting: Revisiting an Underused Diagnostic Tool. *J. Investig. Med.* **2002**, *50*, 214–225. [CrossRef] [PubMed]
7. Lloyd, J.E.; Broughton, A.; Selby, C. Salivary Creatinine Assays as a Potential Screen for Renal Disease. *Ann. Clin. Biochem.* **1996**, *33*, 428–431. [CrossRef]
8. Cooke, M.; Leeves, N.; White, C. Time Profile of Putrescine, Cadaverine, Indole and Skatole in Human Saliva. *Arch. Oral Biol.* **2003**, *48*, 323–327. [CrossRef] [PubMed]
9. Coufal, P.; Zuska, J.; van de Goor, T.; Smith, V.; Gaš, B. Separation of Twenty Underivatized Essential Amino Acids by Capillary Zone Electrophoresis with Contactless Conductivity Detection. *Electrophoresis* **2003**, *24*, 671–677. [CrossRef]
10. Actis, A.B.; Perovic, N.R.; Defagó, D.; Beccacece, C.; Eynard, A.R. Fatty Acid Profile of Human Saliva: A Possible Indicator of Dietary Fat Intake. *Arch. Oral Biol.* **2005**, *50*, 1–6. [CrossRef]
11. Barth, J.A.; Putz, Z.; Vañuga, A.; Velemínsk, J. Radioimmunoassay of Thyroxine in Saliva. *Exp. Clin. Endocrinol. Diabetes* **1985**, *85*, 199–203. [CrossRef] [PubMed]
12. Liu, J.; Duan, Y. Saliva: A Potential Media for Disease Diagnostics and Monitoring. *Oral Oncol.* **2012**, *48*, 569–577. [CrossRef] [PubMed]
13. Chiappin, S.; Antonelli, G.; Gatti, R.; De Palo, E.F. Saliva Specimen: A New Laboratory Tool for Diagnostic and Basic Investigation. *Clin. Chim. Acta* **2007**, *383*, 30–40. [CrossRef] [PubMed]
14. Walsh, L.J. Aspectos Clínicos de Biología Salival Para El Clínico Dental. *J. minim. Interv. Dent.* **2008**, *9*, 59–71.
15. Haeckel, R.; Hänecke, P. The Application of Saliva, Sweat and Tear Fluid for Diagnostic Purposes. *Ann. Biol. Clin.* **1993**, *51*, 903–910.
16. Villiger, M.; Stoop, R.; Vetsch, T.; Hohenauer, E.; Pini, M.; Clarys, P.; Pereira, F.; Clijsen, R. Evaluation and Review of Body Fluids Saliva, Sweat and Tear Compared to Biochemical Hydration Assessment Markers within Blood and Urine. *Eur. J. Clin. Nutr.* **2018**, *72*, 69–76. [CrossRef] [PubMed]
17. Huizenga, J.R.; Gips, C.H. Determination of Ammonia in Saliva Using Indophenol, an Ammonium Electrode and an Enzymatic Method: A Comparative Investigation. *J. Clin. Chem. Clin. Biochem.* **1982**, *20*, 571–574. [CrossRef] [PubMed]
18. Chen, C.-C.; Hsieh, J.-C.; Chao, C.-H.; Yang, W.-S.; Cheng, H.-T.; Chan, C.-K.; Lu, C.-J.; Meng, H.-F.; Zan, H.-W. Correlation between Breath Ammonia and Blood Urea Nitrogen Levels in Chronic Kidney Disease and Dialysis Patients. *J. Breath Res.* **2020**, *14*, 036002. [CrossRef] [PubMed]
19. DuBois, S.; Eng, S.; Bhattacharya, R.; Rulyak, S.; Hubbard, T.; Putnam, D.; Kearney, D.J. Breath Ammonia Testing for Diagnosis of Hepatic Encephalopathy. *Dig. Dis. Sci.* **2005**, *50*, 1780–1784. [CrossRef]
20. Zilberman, Y.; Sonkusale, S.R. Microfluidic Optoelectronic Sensor for Salivary Diagnostics of Stomach Cancer. *Biosens. Bioelectron.* **2015**, *67*, 465–471. [CrossRef]
21. Thepchuay, Y.; Mesquita, R.B.R.; Nacapricha, D.; Rangel, A.O.S.S. Micro-PAD Card for Measuring Total Ammonia Nitrogen in Saliva. *Anal. Bioanal. Chem.* **2020**, *412*, 3167–3176. [CrossRef] [PubMed]
22. Liu, D.; Chen, X.; Zeng, H.; Liu, C.; Tang, B.; Li, Y.; Zhang, L.; Zhang, M. Soap Film as a Rapidly Renewable and Low-Cost Sensor for Detecting Ammonia in Water and Saliva. *Microchem. J.* **2023**, *185*, 108209. [CrossRef]

23. Zilberman, Y.; Chen, Y.; Sonkusale, S.R. Dissolved Ammonia Sensing in Complex Mixtures Using Metalloporphyrin-Based Optoelectronic Sensor and Spectroscopic Detection. *Sens. Actuators B Chem.* **2014**, *202*, 976–983. [CrossRef]
24. Korent, A.; Trafela, Š.; Soderžnik, K.Ž.; Samardžija, Z.; Šturm, S.; Rožman, K.Ž. Au-Decorated Electrochemically Synthesised Polyaniline-Based Sensory Platform for Amperometric Detection of Aqueous Ammonia in Biological Fluids. *Electrochim. Acta* **2022**, *430*, 141034. [CrossRef]
25. Sheini, A. A Paper-Based Device for the Colorimetric Determination of Ammonia and Carbon Dioxide Using Thiomalic Acid and Maltol Functionalized Silver Nanoparticles: Application to the Enzymatic Determination of Urea in Saliva and Blood. *Microchim. Acta* **2020**, *187*, 565. [CrossRef] [PubMed]
26. Monforte-Gómez, B.; Hakobyan, L.; Molins-Legua, C.; Campíns-Falcó, P. Passive Solid Chemosensor as Saliva Point of Need Analysis for Ammonium Determination by Using a Smartphone. *Chemosensors* **2023**, *11*, 387. [CrossRef]
27. Cao, X.; Ding, L.; Xie, Z.; Yang, Y.; Whiteman, M.; Moore, P.K.; Bian, J.-S. A Review of Hydrogen Sulfide Synthesis, Metabolism, and Measurement: Is Modulation of Hydrogen Sulfide a Novel Therapeutic for Cancer? *Antioxid. Redox Signal.* **2019**, *31*, 1–38. [CrossRef]
28. Kimura, H. Physiological Role of Hydrogen Sulfide and Polysulfide in the Central Nervous System. *Neurochem. Int.* **2013**, *63*, 492–497. [CrossRef] [PubMed]
29. Greabu, M.; Totan, A.; Miricescu, D.; Radulescu, R.; Virlan, J.; Calenic, B. Hydrogen Sulfide, Oxidative Stress and Periodontal Diseases: A Concise Review. *Antioxidants* **2016**, *5*, 3. [CrossRef]
30. Rizzo, A.A. The Possible Role of Hydrogen Sulfide in Human Periodontal Disease. I. Hydrogen Sulfide Production in Periodontal Pockets. *Periodontics* **1967**, *5*, 233–236.
31. Feller, L.; Blignaut, E. Halitosis: A Review. *S. Afr. Dent. J.* **2005**, *60*, 17–19.
32. Inoue, S.; Browne, G.; Melino, G.; Cohen, G.M. Ordering of Caspases in Cells Undergoing Apoptosis by the Intrinsic Pathway. *Cell Death Differ.* **2009**, *16*, 1053–1061. [CrossRef]
33. Zaorska, E.; Konop, M.; Ostaszewski, R.; Koszelewski, D.; Ufnal, M. Salivary Hydrogen Sulfide Measured with a New Highly Sensitive Self-Immolative Coumarin-Based Fluorescent Probe. *Molecules* **2018**, *23*, 2241. [CrossRef]
34. Kroll, J.L.; Werchan, C.A.; Reeves, A.G.; Bruemmer, K.J.; Lippert, A.R.; Ritz, T. Sensitivity of Salivary Hydrogen Sulfide to Psychological Stress and Its Association with Exhaled Nitric Oxide and Affect. *Physiol. Behav.* **2017**, *179*, 99–104. [CrossRef]
35. Ahn, B.-K.; Ahn, Y.-J.; Lee, Y.-J.; Lee, Y.-H.; Lee, G.-J. Simple and Sensitive Detection of Bacterial Hydrogen Sulfide Production Using a Paper-Based Colorimetric Assay. *Sensors* **2022**, *22*, 5928. [CrossRef] [PubMed]
36. Cha, J.-H.; Kim, D.-H.; Choi, S.-J.; Koo, W.-T.; Kim, I.-D. Sub-Parts-per-Million Hydrogen Sulfide Colorimetric Sensor: Lead Acetate Anchored Nanofibers toward Halitosis Diagnosis. *Anal. Chem.* **2018**, *90*, 8769–8775. [CrossRef]
37. Carrero-Ferrer, I.; Molins-Legua, C.; Campíns-Falcó, P. Plasmonic Sensor for Hydrogen Sulphide in Saliva: Multisensor Platform and Bag Format. *Talanta* **2022**, *245*, 123449. [CrossRef] [PubMed]
38. Jornet-Martínez, N.; Moliner-Martínez, Y.; Herráez-Hernández, R.; Molins-Legua, C.; Verdú-Andrés, J.; Campíns-Falcó, P. Designing Solid Optical Sensors for in Situ Passive Discrimination of Volatile Amines Based on a New One-Step Hydrophilic PDMS Preparation. *Sens. Actuators B Chem.* **2016**, *223*, 333–342. [CrossRef]
39. Ballester-Caudet, A.; Hakobyan, L.; Moliner-Martínez, Y.; Molins-Legua, C.; Campíns-Falcó, P. Ionic-liquid doped polymeric composite as passive colorimetric sensor for meat freshness as a use case. *Talanta* **2021**, *223 Pt 2*, 121778. [CrossRef]
40. Jornet-Martínez, N.; Hakobyan, L.; Argente-García, A.I.; Molins-Legua, C.; Campíns-Falcó, P. Nylon-Supported Plasmonic Assay Based on the Aggregation of Silver Nanoparticles: In Situ Determination of Hydrogen Sulfide-like Compounds in Breath Samples as a Proof of Concept. *ACS Sens.* **2019**, *4*, 2164–2172. [CrossRef]
41. Konvalina, G. Haick, Sensors for Breath Testing: From Nanomaterials to Comprehensive Disease Detection. *Acc. Chem. Res.* **2014**, *47*, 66–76. [CrossRef] [PubMed]
42. Thepchuay, Y.; Costa, C.F.; Mesquita, R.B.; Sampaio-Maia, B.; Nacapricha, D.; Rangel, A.O. Flow-Based Method for the Determination of Biomarkers Urea and Ammoniacal Nitrogen in Saliva. *Bioanalysis* **2020**, *12*, 455–465. [CrossRef] [PubMed]