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Complete List of Authors:	Lopez Ruiz, Nuria; University of Granada, Electronics and Computers Technology Curto, Vincenzo; Dublin City University, Chemistry Erenas, Miguel; University of Granada, Analytical Chemistry Benito-López, Fernando; Dublin City University, Chemistry Diamond, Dermot; Dublin City University, National Centre for Sensor Research Palma López, Alberto; University of Granada, Capitan-Vallvey, Luis Fermin; University of Granada, Analytical Chemistry

Smartphone-based simultaneous pH and nitrite colorimetric determination for paper microfluidic devices

Nuria Lopez-Ruiz[‡a], Vincenzo F. Curto[‡b*], Miguel M. Erenas[c], Fernando Benito-Lopez[b,d], Dermot Diamond[b], Alberto J. Palma[a] and Luis F. Capitan-Vallvey[c]*

[a] ECsens-CITIC, Department of Electronics and Computer Technology, ETSIIT, 18071, University of Granada, Spain. [b] Insight: Centre for Data Analytics, National Centre for Sensor Research, Dublin City University, Dublin 9, Ireland. [c] Department of Analytical Chemistry. Campus Fuentenueva, Faculty of Sciences, 18071, University of Granada, Spain. [d] CIC MicroGUNE, Goiru Kalea 9 Polo Innovación Garaia, 20500, Arrasate-Mondragón, Spain.

* Corresponding author; email: nurilr@ugr.es; vincenzo.curto2@mail.dcu.ie

‡These authors contributed equally to this work.

Abstract

In this work, an Android application for measurement of nitrite concentration and pH determination in combination with a low-cost paper-based microfluidic device is presented. The application uses seven sensing areas, containing the corresponding immobilised reagents, to produce selective colour changes when a sample solution is placed in the sampling area. Under controlled conditions of light, using the flash of the smartphone as light source, the image captured with the built-in camera is processed using a customised algorithm for multi-detection of the coloured sensing areas. The developed image-processing allows reducing the influence of the light source and the positioning of the microfluidic device in the picture. Then, the H (hue) and S (saturation) coordinates of the HSV colour space are extracted and related to pH and nitrite concentration, respectively. A complete characterisation of the sensing elements has been carried out as well as a fully description of the image analysis for the detection. The results show a good behaviour of a mobile phone as analytical instrument. For the pH, the resolution obtained is 0.04 units of

pH, 0.09 of accuracy and a mean squared error of 0.167. With regard to nitrite, 0.51% at 4.0 mg L^{-1} of resolution and 0.52 mg L^{-1} as limit of detection was achieved.

Keywords: Sensor, Portable instrumentation, Android application, Camera phone, Smartphone, pH, Nitrite concentration, paper-based microfluidic.

1. Introduction

In recent years, lab-on-a-chip (LOC) technology has witnessed growing interest in many different areas, such as life sciences, environmental analysis or health-care. LOC technology constitutes a powerful method in the analytical field providing innovative tools to combine sample pre-treatment, chemical reactions, signal recognition and processing in a single device.[1] Generally, LOC devices must satisfy some design criteria as being inexpensive, accurate, reliable, with low-power consumption, and adaptable to different environmental conditions.[2] These conditions become particularly critical when LOCs are integrated within portable systems for on-site measurements.

Microfluidic analytical devices present some advantages over other analytical methods such as providing reliable measurements with efficiency and speed using small volumes of sample solutions.[3] In this field, paper-based microfluidic devices constitute inexpensive devices whose basis lies on the creation of hydrophilic micro-channels on cellulose or flexible polymeric substrates by means of different hydrophobic patterning materials, such as wax, polymers and inks. Their simplicity allows to carry out many analytical operations[4] such as separation, derivatisation, and even other chemical reactions without external elements, such as multi-step reactions[5] or blood plasma separation in the same device.[6]

Several techniques for the fabrication of paper-based microfluidic devices have been reported in literature[7-10] and, in this work, we make use of our recently developed paper-based microfluidic device, a stamping technique, using filter paper, a PDMS stamp and indelible ink. This one-step fabrication method of fabrication produces microfluidic devices in a reproducible, inexpensive, simple and fast way.[11]

The combination of microfluidic devices with traditional analytical instruments offers an interesting approach for the implementation of cost-effective tools for chemical or

biological analysis.[12-14] Furthermore, the current growth in smartphones and tablets development opens up interesting opportunities for the development of new highly reliable and fast analytical tools.[15-18] These portable devices usually make use of built-in cameras and internal microprocessors to carry out the image-processing, avoiding the need of external computers or elements, and providing fast and accurate results.[19-21] Moreover, the connectivity of mobile phones allows to send data easily to a network platform in order to share information and providing real-time results at the point of need.[16,22] In this context, several works combining the advantages of paper-based microfluidics and mobile phones have been already reported in literature.[23-25]

In some fields, microfluidic devices make use of colorimetric sensors. When the sample solution reaches the test zones with the reagents precisely positioned, the desired reaction takes place, giving a colour change.[26] Moreover, there is a trend towards the use of colorimetric sensors arrays that, usually, consists of a combination of low-selective sensors to obtain cross-information for several analytes in a single experiment.[27,28] In most of these cases, colour changes are quantified using colour components of different colour spaces. The colour can be determined by using specific instruments, such as digital colour detectors[28], commercial colorimeters[29,30], photodetectors[31,32], or spectrophotometers[33], or using imaging devices, such as digital CCD and CMOS digital cameras[27,34,35], scanners[36,37], and, in the last years, built-in smartphone cameras.[38]

The present work demonstrates a paper-based microfluidic colorimetric sensor for simultaneous determination of pH and nitrite concentration in water samples. The determination of nitrite in drinking water is necessary not only because of its undesirable character, approximately ten-fold more toxic than nitrate[39], but also because it acts as an indicator of bacterial contamination. In fact, the European Community requires that water for human consumption should have a maximum admissible concentration for nitrite of 0.5 $mg \cdot L^{-1}$ moreover, the World Health Organisation guideline value is 3 $mg \cdot L^{-1}$.[40] Additionally, the drinking water standard for pH is between 6.5 and 9.5 pH.

In previous studies in our groups, we developed portable instrumentation for determination of pH in solutions by using an array of non-specific colorimetric sensors and digital colour detectors.[28] Also, we developed paired emitter-detector diode systems to obtain nitrite

concentration or pH by measuring the time of discharge of the reverse biased light emitting diode (LED) acting as detector.[13,41] Here, a picture of a paper-based microfluidic device is taken with a smartphone camera and later processed, in order to extract the colorimetric information and use it for determination of pH and nitrite levels in water.

Both pH and nitrite sensitive membranes are combined in a single disposable multi-sensing device, in which hue and saturation coordinates of the HSV colour space are used for the determination. The analysis of the colour information is performed through a smartphone, which is capable of carrying out a two-dimensional study of the harvested pictures of the multi-sensing devices using a developed Android application for image-processing. The algorithm has been optimised to avoid any detection interference such as wrong positioning of the paper-based sensor or the influence of different light sources when a picture is taken. Moreover, the only used light source is the built-in flash of the smartphone camera to avoid the need of any other external elements and simplify the operational procedure.

2. Experimental

2.1. Reagents and materials

For the fabrication of the microfluidic device, according to the process explained in our recent publication,[11] standard laboratory filter paper Whatman© paper grade 1 and indelible ink Lumocolor© Permanent Universal Black Ink from Staedtler (Staedtler Mars CmbH & Co. KG., Germany) were employed. The viscosity of the ink was reduced using a 10:1 *v/v* ink-solvent mixture, where the solvent is a 1:1 *v/v* ethanol/*n*-propanol solution.

The chemicals used to prepare the pH sensitive membranes were hexadecyltrimethylammonium bromide (CTAB, CAS No. 57-09-0) and phenol red (CAS No. 143-74-8) purchased from Sigma (Sigma-Aldrich Química S.A., Spain), chlorophenol red (CAS No. 4430-20-0) from Merck (Merck Millipore, Germany) and sodium hydroxide (CAS No. 1310-73-2) from Panreac (Panreac Química S.A., Spain). For the preparation of nitrite sensitive areas, the chemicals used were Nafion perfluorinated resin solution (CAS No. 31175-20-9), sulphanilamide (CAS No. 63-74-1) and *N*-1-napthylethylenediamine dihydrochloride (NED) (CAS No. 1465-25-4) from Sigma and polyethylene glycol 400

(PEG, CAS No. 25322-68-3), 2-propanol, and citric acid (CAS No. 77-92-9) were purchased from Panreac.

For calibration and validation of the device aqueous solutions of known pH and nitrite concentrations were prepared. Phosphate buffer solutions were prepared by mixing suitable amounts of NaH_2PO_4 (CAS No. 10049-21-5) and Na_2HPO_4 (CAS No. 7558-79-4), both from Panreac. All aqueous solutions were made using reverse-osmosis type quality water (Mili-RO 12 plus Milli-Q station from Millipore, resistivity 18.2 MΩ·cm). A Crison pH-meter (Crison Instruments, Barcelona, Spain, model Basic 20) with a combined double junction glass electrode, calibrated against two standard buffer solutions (pH 4.0 and 7.0), was used for the pH measurements.

2.2. Fabrication and preparation of the paper-based microfluidic devices

The fabrication process of the paper-based microfluidic devices was performed accordingly to our early proposed contact stamping technique.[11] After the process, a PDMS stamp is obtained and ready to be used for contact stamping using a 10:1 *v/v* ink-solvent mixture. This is followed by contact stamping on Whatman grade 1 paper, in which the transferred indelible ink on paper defines the microfluidic contours.

The final design of the device (Figure 1) consists of one main central area (sampling area), seven sensing areas with independent channels, one blank for reference during the colorimetric detection and two marks (a triangle and a square). Each sensing area is a circle with an internal diameter of 6 mm and an outer diameter of 8.2 mm, while the diameter of the central area is of 9.6 mm. The designed length and width of the channels is 6 mm and 1.7 mm, respectively. The entire size of the designed microfluidic device is 35.3 mm × 50 mm. The two marks are included in the design with the aim of allowing the alignment between the microfluidic device and the smartphone camera during the image-acquisition procedure, as it will be explained in the following section. In addition, it is worth noting that the final dimensions of stamped microfluidic devices may vary from the designed ones, according to our previous observations.[11]

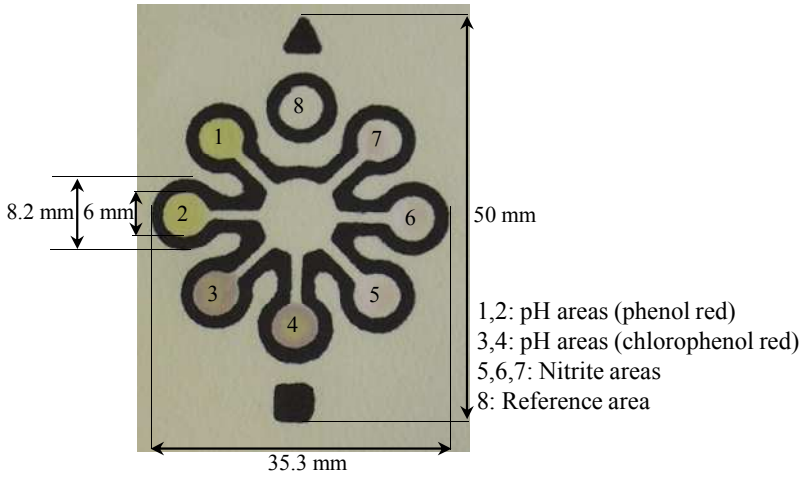

Figure 1. Microfluidic device with the reagents placed in each sensing area.

In order to perform a colorimetric detection over each area, the pH sensing areas (1 to 4 in Figure 1) were prepared with conditions of no leaching, tonal colour change by reaction, and covering the pH range of interest (from 4 to 9) using two different pH indicators: phenol red and chlorophenol red. This pH range was selected to cover the usual drinking water standards in most regulations[42]. Additionally, the nitrite sensitive areas (5 to 7 in Figure 1) were designed to provide no leaching, and saturation colour change based on Griess reaction[43], instead of tonal colour change as in the case of pH, since the reagents for nitrite determination are uncoloured. Therefore, this paper-based microfluidic device allows simultaneous detection of pH and nitrite, with replicates of each type of sensor in a single device.

Each sensing area was prepared by drop casting of the corresponding reagents under ambient atmospheric conditions. Two different pH indicators solutions were prepared by dissolving 1.77 mg of phenol red, 8.2 mg of CTAB, and 5 µL of NaOH (0.1M) in 5 mL of water, and, 3.17 mg of chlorophenol red and 10.93 mg of CTAB in 5 mL of water. One drop of 1.5 µL was cast for the red chlorophenol cocktail while two drops of 1.5 µL were needed in the case of phenol red indicator. Both cocktails for pH include an ammonium quaternary salt (CTAB) to avoid leaching by basic form of indicators forming ionic pairs. For the preparation of the nitrite sensing areas three different solutions were needed: solution (1) made of 40 % of Nafion, 33.3 % of 2-propanol and 26.7 % v/v of water containing 0.013 $g \cdot mL^{-1}$ of PEG; solution (2) with 33.2 mg of sulphanilamide and 126.8

mg of citric acid dissolved in 1 mL of water; and, finally, solution (3) made of 2.32 mg/mL of NED. The latter was mixed with solution (2) in 1:1 *v/v* prior to use. Subsequently, 1.5 μL of each solution (1) and (2)+(3) were casted in the sensing areas for nitrite determination. It is worth noting that between consecutive castings of the reagents cocktails it is important to let the device dry to avoid reverse flow of the reagents into the channel. After deposition of the sensing membranes, the support was left to dry in darkness, as the NED is photosensitive, for at least 5 minutes before being used. Nafion was included in the nitrite cocktail to avoid leaching of cationic *azo*-dye formed by reaction. After the described process, the obtained membranes appear coloured for the pH areas and colourless for the nitrite regions as shown in Figure 1.

2.3. Measurement protocol

To activate the reactions that produce colour changes in the sensing areas, 30 μL of a solution of known pH and nitrite concentration for calibration and unknown values for validation were dropped in the central sampling area of the microfluidic device. Due to the capillarity of the filter paper and the barriers created by the stamped indelible ink, the solution flowed towards the seven sensing areas where the corresponding reagents were placed. Fifteen minutes were needed to complete the reaction on each sensing area and leave to dry the microfluidic device in order to obtain a uniform and stable colour that can be easily detected by the Android application installed on the smartphone. The whole process had to be carried out in a dark environment since the NED is photosensitive and it acquires a purple colour when it is exposed to ambient light. Moreover, it is important to take into account that the device must be kept in horizontal position and over a non-absorbent surface. In this way, the flow of solution is not favoured in any direction and there is no absorption of reagents or sample solution to the supporting material below the device. However, the microfluidic device can be also sandwiched between transparent adhesive plastic tape in order to guarantee homogenous flow in all the directions of the device and avoid contaminations.[10]

Once all the sensing areas were allowed to react, a picture of the microfluidic device is captured using the developed Android application, installed in a Samsung Galaxy SII smartphone, as explained in the next section. The picture has to be taken under controlled

illumination conditions, since the detection using colorimetric devices needs a constant light source over the microfluidic devices during the measurement process[16,44] or some kind of compensation procedure to remove the influence of the ambient light.[45,46] In this case, there is only a light source for the system which is the flash of the built-in smartphone camera.

3. Developed software for image-processing

Before the development of a mobile phone application, it has to be taken into account the operative system that the application should supports. In this case, Android was selected as operating system due to the easy of programming and the advantage of being free license against other proprietary operating systems such as iOS (Apple Incorporation, California, USA) or BlackBerry OS (BlackBerry, Ontario, Canada). Moreover, Android currently leads the smartphone operative system market with almost a 75 % of the devices while iOS has only the 17%.[47] In this work, a Samsung Galaxy SII (Samsung, South Korea) was used as detection platform. This smartphone has a dual core processor, runs the Android version 4.1.2, and has a built-in camera of 8 megapixels which includes a LED flash, used as light source. The dimensions of the phone are 125.3 × 66.1 × 49 mm and it has a display of 4.3" with 640 × 480 pixels of resolution.

Camera parameters were fixed in the developed application in order to avoid automatic configurations of the mobile phone. The International Organisation for Standardisation (ISO) parameter determines the sensitivity of the image sensor in relation to the environmental light. Since in this case, the built-in flash provides favourable illumination conditions for colorimetric detection in a dark environment, the ISO was set to 200 in a range from 100 to 800. This allows obtaining almost noise-free pictures, compared to pictures taken at higher ISO due to the increased sensitivity of the camera detector. The focus mode was set to macro, considering that the distance between the camera lens and the sensor device is less than eight centimetres. Also, the application uses the autofocus option, which avoids capturing blurred and out of focus pictures of the microfluidic device. The resolution of the pictures was set to the higher available value in the Samsung smartphone, which is 3264 × 2448 pixels to guarantee a high number of pixels for each sensing area for

the image analysis. However, on the other hand, the time of processing was also increased, being of fifteen seconds for this resolution. All the pictures were saved in JPEG (Joint Photographic Experts Group) format as it is the default format in most of the smartphones, with a resolution of 96 dots per inch (dpi) and 8-bits per RGB channel (24 bits in total).

The flash was configured in torch mode, with the aim of providing a constant light source during the image acquisition process. The spectrum of the emitted flash light has the main component placed at the wavelength of 440 nm, which provides a bluish illumination. The white balance was then set to daylight mode (5500K of colour temperature) to match the colour temperature of the light emitted by the LED flash in the smartphone (5500K-6000K).

Figure 2 displays four different screenshots of the developed application at different stages in the image-acquisition procedure. The main menu of the application (Figure 2a) is customised to offer the possibility of capturing a new picture of the microfluidic device or analysing a previous saved picture from the gallery. When a new picture is required, the application, with the fixed camera parameters, starts the capture process turning on the flash light. Then, two red marks are displayed over the camera view (Figure 2b) in order to align the two corresponding marks of the microfluidic device with them. This step allows taking pictures always with the same orientation of the devices, ensuring that the user does not place the sensor device in a wrong position, and simplifying the processing of the picture. When the picture is taken or loaded from the gallery, the application turns off the flash light to avoid long exposure time of the nitrite light-sensitive areas and detects the eight sensing areas using the image-processing later explained, and shows the detected areas to the user (Figure 2c).

It is worth noting that during the acquisition of the picture, the smartphone needs to be maintained in parallel position with the microfluidic device in order to avoid distortions in the shape of the sensing areas due to the inclination of the mobile. If the smartphone is not correctly placed, the sensing areas will be distorted in the image preview and the detection of the areas will not be carried out correctly. In such a case, the user could discard the picture to take a new image by simply press the ‘Redo’ button at the bottom right (Figure 2c) and modify the position of the phone. Once the user agrees with the detected sensing

areas, the 'Process' button allows the smartphone to go over the next step to determine the nitrite concentration and the pH of the sample. The final results are displayed as shown in Figure 2d, allowing the user to save the final results on the phone memory.

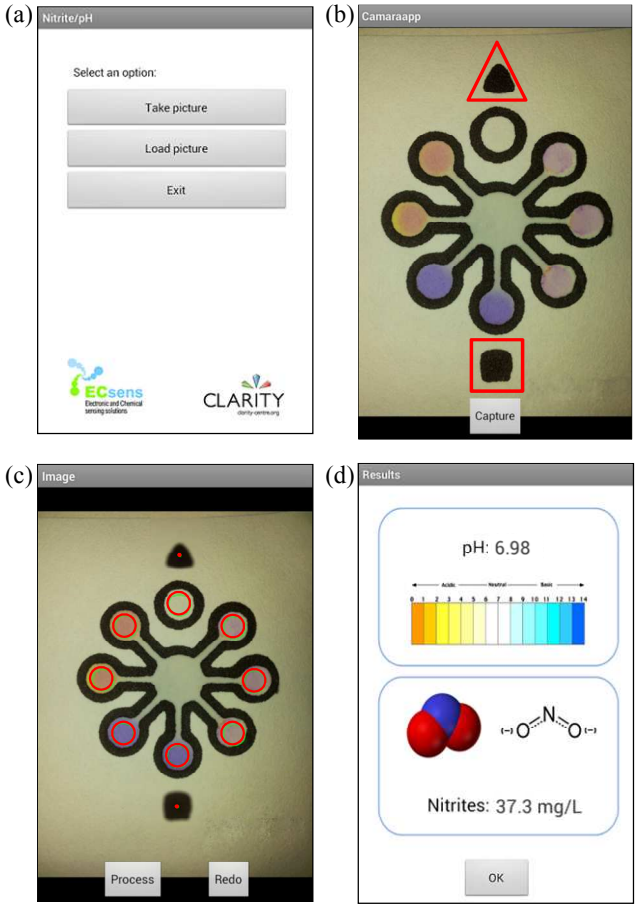

Figure 2. Screen captures of the developed Android application: a) main menu, b) acquisition process, c) marks and sensing areas detection and d) final results obtained after image processing.

3.1 Image processing

Although Android has its own functions to extract colour information directly from JPEG images without any decode algorithm, in this work an open source computer vision library (OpenCV) was integrated within the Android application. This permits to carry out in a more effective way the image processing needed for the detection of the sensing areas. Since OpenCV is focused and optimised for real-time image-processing, the response using this library is normally faster than using Java libraries. In Figure 3, a scheme of the image-

processing process carried out by the developed application is shown. The analysis was performed in three different stages: detection of the marks, detection of the sensing areas and, finally, colour analysis of each sensing area in order to relate the information to the values of pH and nitrite concentration.

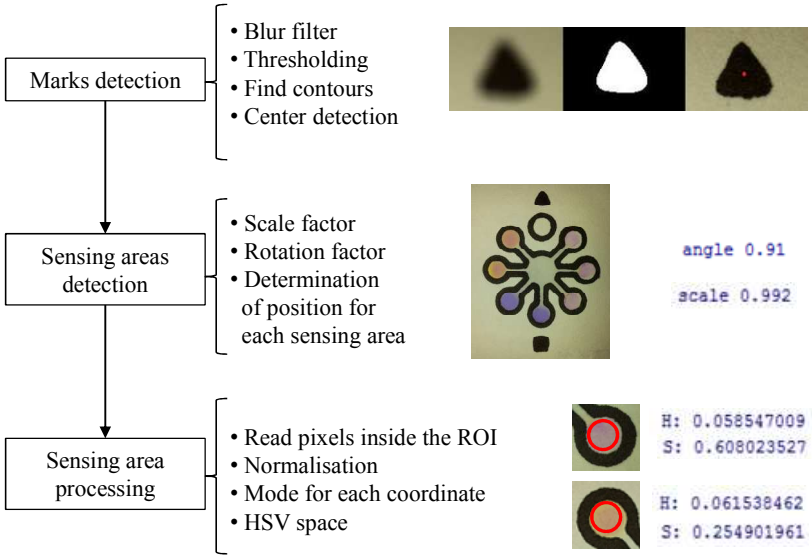

Figure 3. Steps of the image-processing carried out for each picture of the microfluidic device.

For the detection of the marks, firstly, a blur filter was applied in order to attenuate the thinner dark areas in the picture, such as the edges of the microfluidic device, and keep dark the wider areas[48], such as the marks. The filter was applied pixel by pixel using the kernel (k) described by the Eq.1 with a size of 40×40 for the mask. After the smoothing, an inverse thresholding was applied to contrast the darker areas, which corresponds to the marks, from the rest of the pixels. A binary mask was obtained, as shown in Figure 3, and, by using a predefined function of OpenCV, it was possible to define the contours of the marks to determine the centre position of the marks.

$$k = \frac{1}{40\times40} \cdot \begin{bmatrix} 1 & \cdots & 1 \\ \vdots & \ddots & \vdots \\ 1 & \cdots & 1 \end{bmatrix} \qquad \text{(Eq.1)}$$

Although the microfluidic device is placed in the same position during the acquisition procedure (Figure 2b), light variations in the angle or the scale could appear between

different images. In order to correct them, and determine the correct positions of the sensing areas, affine transformations were applied to the matrix of each red, green and blue channel. With the position of the marks calculated, and knowing the true distance between the two symbols, the factor or scale and rotation can be calculated over the taken picture, as shown in Figure 3. To correct the factors, the matrices of rotation (R) and scale (S) shown in Eq.2 and Eq.3 are applied to the each channel.[49]

$$R = \begin{bmatrix} \cos\alpha & sen\,\alpha \\ -sen\,\alpha & \cos\alpha \end{bmatrix} \quad \text{(Eq.2)}$$

$$S = \begin{bmatrix} s & 0 \\ 0 & s \end{bmatrix}, \quad \text{(Eq.3)}$$

where α represents the angle of rotation and s is the scale factor previously calculated using the real distances in the pictures.

Finally, the position of each sensing area was determined taking into account the distances to the corrected position of the marks. Then, with the centre of the sensing element determined, the pixels inside the area were read one by one in order to extract their RGB coordinates. In order to correct intensity variations of light in the picture, each coordinate was normalised by the coordinates of the reference sensing area following the Eq.4,[33,45] where n is equal to 8, corresponding to the number of bits used by the mobile phone to encode each colour channel. Then, the HSV coordinates were determined from the RGB coordinates,[50] and the mode of the hue value for each sensing area provided the necessary information to relate to the pH and the nitrite concentration of the sample.

$$RGB_{normalized} = 2^n\, RGB_{acquired}/RGB_{white} \quad \text{(Eq.4)}$$

Since there are two sensing areas for each indicator and three for the nitrite determination, the average value is provided as the final hue and saturation values to calculate the pH and the concentration of nitrite, respectively.

4. Results and Discussion

4.1. pH determination

A study of the time required by the pH indicators to complete the colorimetric reaction in the sensing areas was carried out. That time comprises from when the sample to be

analysed is placed in the central area of the microfluidic device until the capture of the picture. It had to be well defined for all the experiments since the colour in the sensing area must be uniform, and, besides, the HSV coordinates take different values when the area is wet or dry. For this purpose, four and three sensing areas were modified using phenol red and chlorophenol red reagents, respectively, in one single paper device. For each pH value a different device was employed and measured at different time interval (from 5 to 20 minutes with 5 minutes step). As can be seen in Figure 4, the behaviour in the chlorophenol red was similar for different times, but phenol red needed 15 minutes to obtain a stationary response. Therefore, 15 minutes was selected as the optimum time before taking the picture.

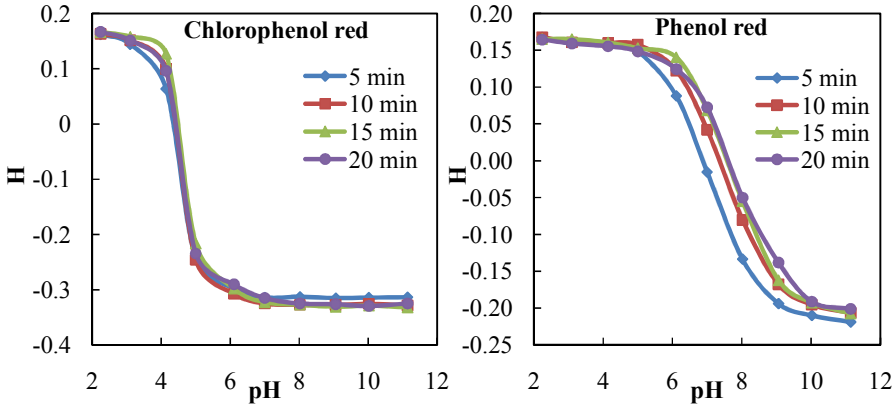

Figure 4. Response of the pH indicators for chlorophenol red (n=3) and phenol red (n=4) as function of the reaction time.

The responses of each pH indicator for 15 minutes of reaction were studied by depositing in the sampling area of the microfluidic device the solutions of different pH values from 2 to 12 and using 15 replicates for each indicator at each pH value. As can be seen in Figure 5, the chlorophenol red presents a colour transition from yellow to purple between pH 4 and 6, while the phenol red changes colour from yellow to pink from pH 6 to 9, approximately. In this way, using these two pH indicators the pH range of interest for drinking water, between 4-9 of pH, can be analysed and determined. With regard to the standard deviation, the resulting error bars are too small to be visualised in the final graphs. However, the calculated mean standard deviation values in the range of interest for each pH indicator were 0.01 and 0.009 for chlorophenol red and phenol red, respectively.

As two indicators were employed, it was necessary to identify which one has to be taken into account according to their ranges of variation. For an unknown value of pH, the measured hue value of each indicator was different and only one of them is changing colour within the range of interest, determined by variations of the hue coordinate for each pH indicator. For instance, typical changes of hue coordinate are from -0.3 to 0.15 for chlorophenol red and from -0.25 to 0.2 for phenol red, as can be seen in Figure 4. Therefore, by measuring the hue coordinate of the two pH indicators, it can be determined which one is within the range and, therefore, which indicator must be considered for the final pH determination. The curves were fitted by Eq.5 and Eq.6 for the chlorophenol red and phenol red, respectively, in order to provide an accurate pH value in the range of interest for each indicator, as shown in Figure 5.

$$H = -0.0193 \cdot pH^3 + 0.4045 \cdot pH^2 - 2.825 \cdot pH + 6.253 \quad (R^2 = 0.9984) \qquad \text{(Eq.5)}$$

$$H = -0.1124 \cdot pH + 0.8431 \quad (R^2 = 0.991) \qquad \text{(Eq.6)}$$

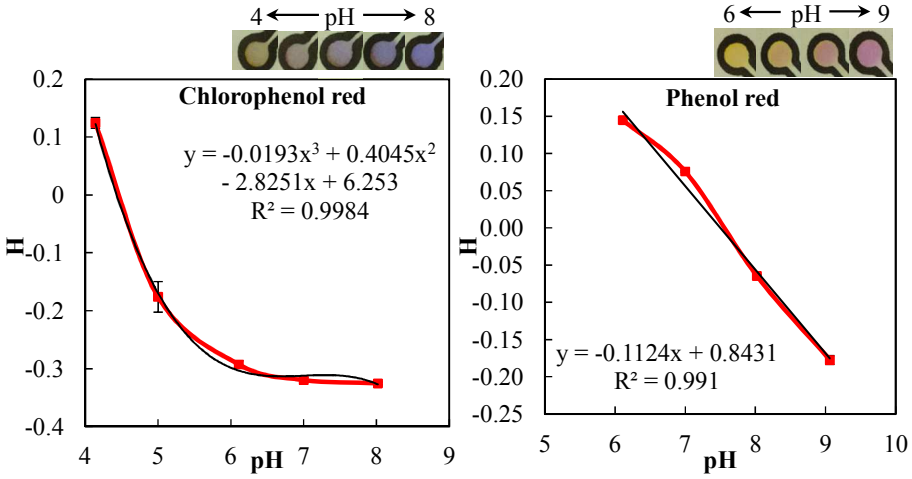

Figure 5. Fitting curves in the regions of interest for the pH indicator.

4.2. Nitrite determination

For the determination of nitrite concentration between 4.0 and 85.0 mg·L^{-1}, the saturation coordinate S of the HSV colour space provided the most accurate colorimetric information, due to the colourless form of the Griess reagents used in this study. As depicted in Figure 6, the concentration of nitrite can be related to the inverse value of saturation coordinate. For each nitrite concentration fifteen replicates were taken at room temperature (21 ºC), using

the three nitrite sensing areas of five different paper microfluidic devices. The curve shows an increasing in the saturation value when the nitrite concentration increases. The logarithm of the curve can be fitted to a linear function of the form $y=ax + b$, where a = -0.596 and b = 1.583 for this case, with a correlation factor R^2 equal to 0.994.

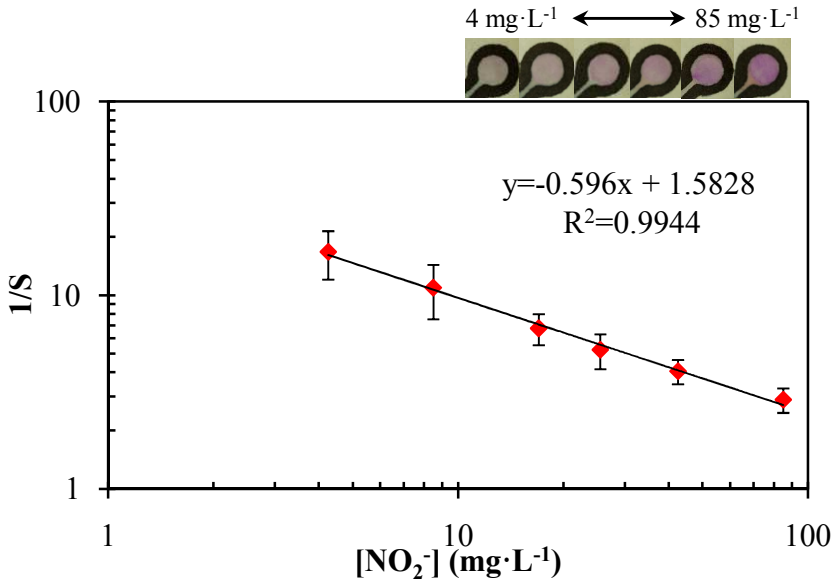

Figure 6. Fitting curve for nitrite concentration determination (n=15).

Therefore, taking into account the curves obtained for each sensing membrane used in the microfluidic devices, the pH and the nitrite concentration of a liquid sample can be determined.

4.3 Validation

In order to test the reliability of the smartphone as a chemical detector, nine solutions with different concentration of nitrite at different pH were prepared (three different nitrites concentrations at three different values of pH). 30 µL of each sample solution were placed in the centre area of the microfluidic device and, after 15 minutes, a picture was taken with the smartphone. For each solution three replicates were taken using three different microfluidic devices. Since in the same device there are replicates for each cocktail, 18 replicates for pH were taken while for nitrite were 27 replicates in total.

For the pH determination, the mean square error (MSE) obtained was 0.02 (Table 1), which shows that the developed application and the prediction models are suitable for pH measurement.

Table 1. Comparison between the obtained pH using the potentiometric and the smartphone method.

Potentiometric pH value	Smartphone pH value
6.00	6.12
6.07	6.02
7.09	7.20
8.08	8.05
8.15	8.08

The accuracy obtained as the standard deviation of the validation data was 0.09 units of pH. To determine the resolution of this system, it has to be taken into account that the H parameter is a linear combination of the RGB coordinates, which are represented by 8 bits each one. Assuming the worst case scenario with only 6 significant bits, and a range of H from 0.15 to -0.33 as shown in Figure 5, the resulting resolution of pH is 0.04.

For measuring nitrite concentration, the results obtained were less accurate than for the pH due to the observed inhomogeneity of the sensing areas after the reaction. When the membrane is deposited on the corresponding area, the paper did not always absorb the sample in the same and uniform directions due to the membrane composition. In particular, the hydrophobic character of Nafion modifies the wetting properties of the paper and the reaction does not always occur in a uniform way. This fact makes difficult the detection of the HSV coordinates despite of using the mode value, since slightly variations of the S coordinate imply a higher variation of the determined concentration value. For the calculus of the technical specifications in the nitrite determination, the corresponding potential fit shown in Figure 6 is considered.

$$\frac{1}{S} = 38.264[NO_2^-]^{-0.596} \qquad \text{(Eq. 7)}$$

To obtain the resolution, it is necessary to take derivatives in both sides and approximate these derivatives to increments. The resolution is then given by:

$$\Delta[NO_2^-] = \frac{\left(\frac{1/S}{38.264}\right)^{-1/0.596}}{-0.596\cdot\left(\frac{1}{S}\right)} \cdot \Delta\left(\frac{1}{S}\right) \quad \text{(Eq. 8)}$$

where $\Delta(1/S)$ is related to the resolution of the smartphone, ΔS which in this case is 8 bits, as shown in this equation.

$$\Delta\left(\frac{1}{S}\right) = \frac{-1}{S^2} \cdot \Delta S \quad \text{(Eq.9)}$$

Taking this into account, the value of resolution depends on the concentration, and its value is 0.51% at 4.0 mg·L^{-1} of nitrite and 1.03% at 42.5 mg·L^{-1}. The limit of detection (LOD) of the system was obtained using the standard criteria LOD = $y_b + 3s_b$, where y_b is the average of the blank signal and s_b is the standard deviation of the blank determined using nine replicates. With this criterion, the value of LOD is 0.52 mg·L^{-1}, an acceptable value taking into account the drinking water standard value of 0.5 mg·L^{-1}.

4.4 Discussion

Smartphones cameras are generally designed to be used in a wide variety of lighting conditions and their response is mainly influenced from the ambient light sources. This is, in fact, one of the main limiting factor for their employment as analytical instruments. One of the main strategies to get around this problem is the employment of external light sources with constant conditions of illumination and 3D-printed enclosures in which samples are fitted in a determined position in order to carry out colorimetric detection. [17,19,33,38] In the present work, it was not necessary to employ any custom-made enclosure to place and keep held the microfluidic device. Here, the built-in flash of the camera phone acted as light source during the acquisition procedure which can be performed in relative controlled ambient light conditions. Nevertheless, the employment of a dark environment was required to avoid photo-bleaching of the photosensitive NED before the testing of the sensor and, also, to minimise the influence of ambient light.

As mentioned in the image-processing section, the implemented smartphone application is able to minimise the influence caused by the wrong positioning of the microfluidic device during the acquisition picture process using two marks in the design of the device. The

rotation and scale factors of the taken picture are obtained in order to correct eventual misalignments that can influence the detection of the seven sensing areas. Therefore, it is possible to perform multi-detection of the areas in one single experiment, using one single microfluidic device without need of external elements for the processing (*i.e.*, computers[22,25], colorimeters[16,32], or scanners[23]), avoiding the influence of external lights, and with no need at all to plug any additional feature to the phone. Moreover, the two marks shown on the screen of the app when in acquisition mode (Figure 2b - camera and LED on) also provide an easy-to-use alignment tool between the paper microfluidic device and the mobile phone camera before a picture is harvested. We believe that all the developed features in the application reduce considerably the time of response of the system, the complexity of the procedure, and the cost of the performed analysis as well. Furthermore, due to the colorimetric nature of the produced reactions it is not necessary the use of external optical excitation of the membranes or samples to obtain the response of interest.[18,21] For these reasons, the system here presented is a simple, easy to use and inexpensive analytical instrument.

Moreover, the application can be exported to other Android devices, which is an advantage over previous developed works using other operative systems for mobile phones.[15,19] In fact, the Android application here developed has been also tested in other smartphone such as the Samsung Galaxy S2 similar to the used for calibration purposes. Despite of being the same model, the illumination produced by the LED flash emitted by each smartphone is different. They present differences mainly in the green and red wavelengths regions of the spectra. However, using the normalisation procedure previously developed in this work, the light source factor is balanced out during the processing of the picture. Taking this into account, the results obtained for pH determination with the second device show an error of 0.28 and 0.07 units of pH for phenol red and chlorophenol red indicators respectively and, with regard to measurement of nitrite concentration the mean error obtained is 1.98 $mg \cdot L^{-1}$ from the values measured with the original smartphone.

5. Conclusion

A smartphone application for Android has been developed and tested using a paper-based microfluidic device for the simultaneous determination of pH and nitrite concentration. The

application studied the change of the hue and saturation coordinates of the HSV colour space for different sensing areas by using a customised algorithm for the image-processing over a picture taken with the built-in camera.

After the measurement of the analytes, using the smartphone as capture device and colour detector from a picture of the microfluidic device, the results showed a good performance of the smartphone as analytical portable instrument under moderate controlled conditions of light. The sensing membranes were fully characterised and tested in the range of interest. From the analysis, with regard to the pH measurement, the MSE was 0.167, the accuracy 0.09 and the resolution 0.04 units of pH. In the case of nitrite concentration determination, despite the non-homogeneity of the sensing membranes, the values of resolution and LOD are 0.51% at 4.0 $mg \cdot L^{-1}$ of nitrite concentration and 0.52 $mg \cdot L^{-1}$ respectively. The application has been tested in a second smartphone, obtaining similar results, despite the different light source used during the measurement process. Therefore, the developed algorithm removed the influence of the light source and the positioning of the device for taking the image. Despite of these facts, correction algorithms are currently being studied and optimised in order to completely remove the influence of the light source for different models of smartphone which are suitable to be used as chemical detectors.

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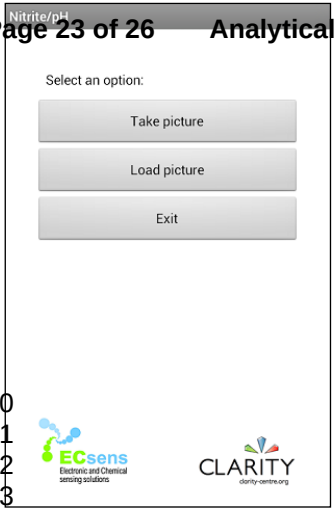
(a)



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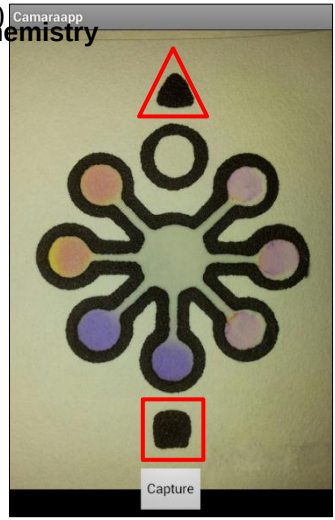

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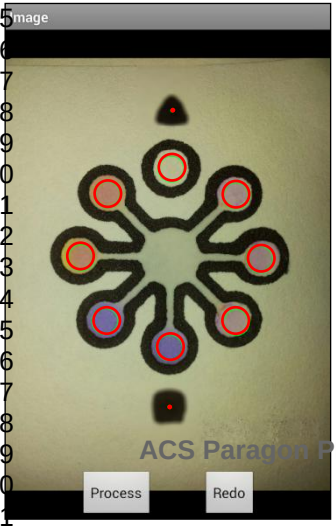

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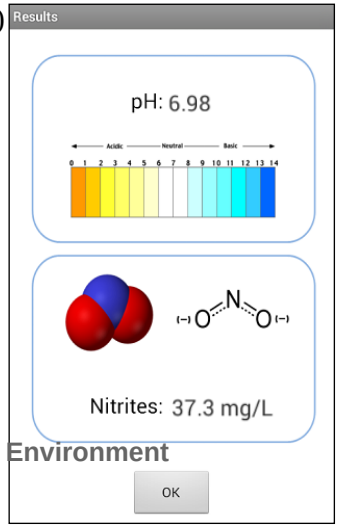

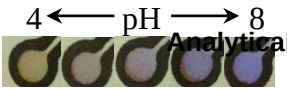

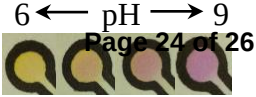

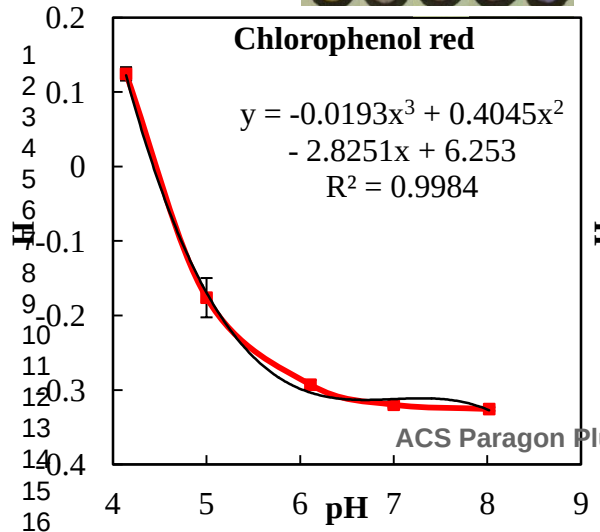

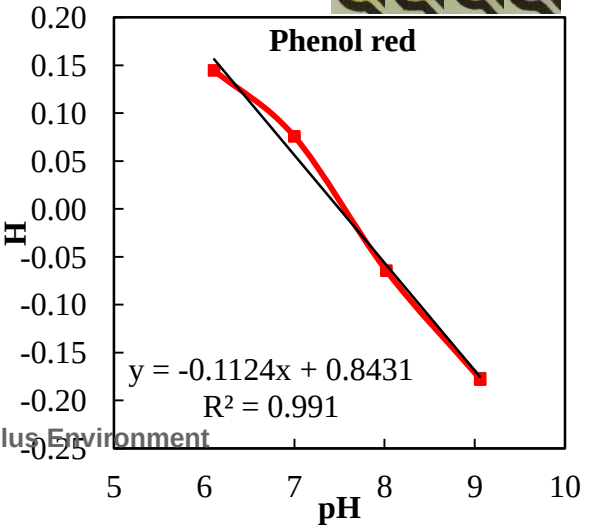

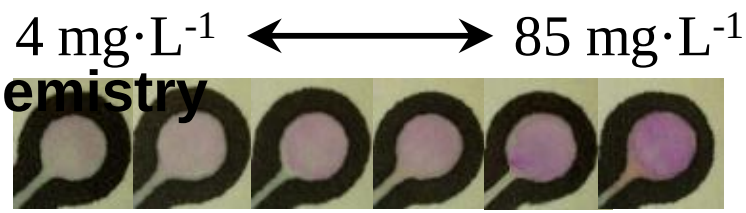

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R^2=0.9944

1/S

100

10

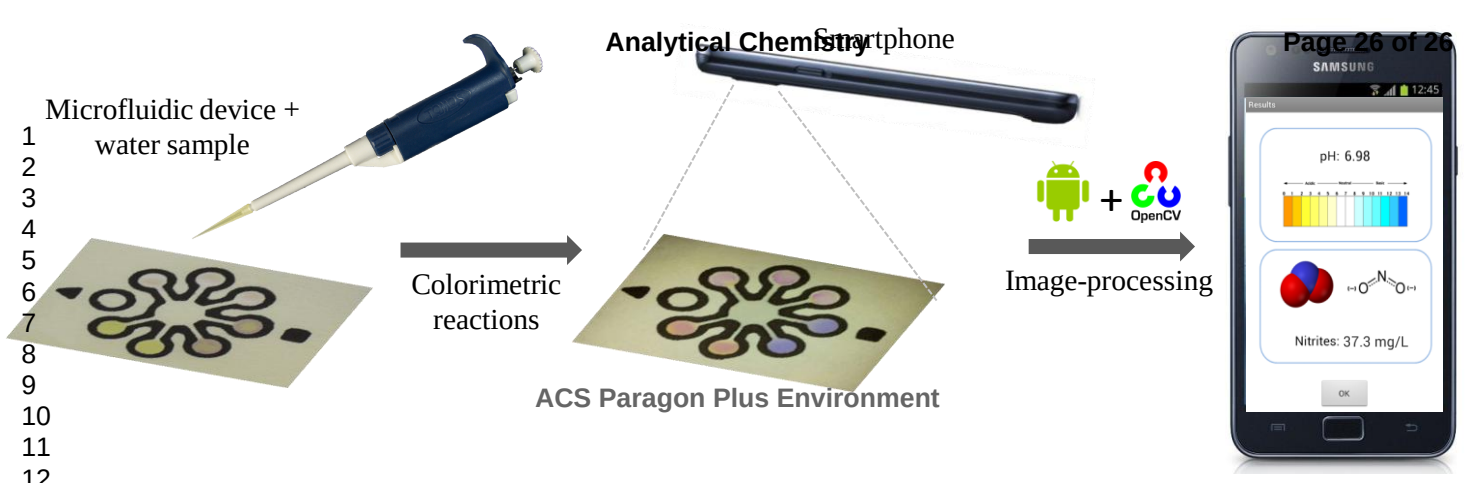
1

1

10

100

$[NO_2^-]$ (mg·L^{-1})


Microfluidic device +
water sample
Colorimetric
reactions
Smartphone
ACS Paragon Plus Environment
+
OpenCV
Image-processing
SAMSUNG
12:45
Results
pH: 6.98
Nitrites: 37.3 mg/L
OK