Accepted Manuscript

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Please cite this article as: Isabel M. Perez de Vargas Sansalvador, Nuria Lopez Ruiz, M.M. Erenas, Luis Fermin Capitan Vallvey, Simon Coleman, Dermot Diamond, Maria Dolores Fernandez Ramos , Towards an autonomous microfluidic sensor for dissolved carbon dioxide determination. The address for the corresponding author was captured as affiliation for all authors. Please check if appropriate. Microc(2017), doi:10.1016/j.microc.2018.02.018

Towards an autonomous microfluidic sensor for dissolved carbon dioxide determination

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Abstract

In this work, we present a new system for the determination of dissolved carbon dioxide (from 7.2 ppm to 425.6ppm) in aqueous environments. Microfluidic technology has been incorporated in sensor design to reduce the volume of samples and reagents. Moreover, a detection system has been integrated in the chip, consisting of a white light-emitting diode as a light source and a high-resolution digital colour sensor as the detector, which are able to detect changes in colour produced by the reaction of the sensing chemistry and carbon dioxide in water.

The optimised parameters found for the system are: flow rate 0.6 mL·min^{-1}, integration time 30 s and the time for pumping of solutions was 3 min, obtaining a LOD of 7.2 ppm. The CO_2 response, reproducibility, precision, and stability of the sensing chemistry have been studied and compared with those obtained using bench-top instrumentation (i.e. a spectrophotometer), obtaining good agreement.

1. Introduction

Microfluidic technology has allowed the miniaturization of analytical systems, minimising the quantity of samples, reagents, and power consumption needed for analysis [1]. The challenge now is to simplify the whole analysis system, avoiding the necessity for specialised training. Several studies can be found in the literature investigating alternatives to classic analytical methods, for instance, with the use of portable devices [2-6] or the novel usage of smartphones as detectors [7-9]. In this way, laboratory costs are reduced and there is no need for sample transportation. In order to achieve an autonomous system, microfluidic technology has been used together with low-power detection systems. In addition, the design of new systems that are portable and deployable has been possible with the use of photodiodes and light-emitting diodes (LEDS) as detectors and light sources, respectively, together with syringe pumps. [10, 11].

CO_2 determination is of great importance in many environmental, clinical, biological, and industrial fields [12-15]. Measurement of the concentration of dissolved carbon dioxide is also extremely interesting in the fields of hydrology, biogeochemistry,

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ecology, and others [16]. It is also a parameter of interest for questions related to the terrestrial–aquatic connectivity of groundwater and surface water [17], and the carbon cycle in general [18]. This also applies to subsurface saturated and unsaturated (vadose) zones in the terrestrial environment. Dissolved carbon dioxide plays an important role in aquatic plant life and fish as they depend upon carbon dioxide in water for their growth. Microscopic plant life suspended in the water (phytoplankton), as well as large rooted plants, utilize carbon dioxide in photosynthesis. The quantity of carbon dioxide increases when the oxygen concentration in waters containing organic matter is reduced. This scarcity of oxygen makes it more difficult for fish to breath and hence to survive [19]. Oceans play an important role in climate regulation. The uptake of anthropogenic CO_2 since 1750 has led to the ocean becoming more acidic, with an average decrease in pH of 0.1 units [20]. Therefore, it is important to understand the dynamics of the oceanic carbonate system to study ocean acidification and its consequences [1].

Scientists are making great efforts to include miniaturized and integrated detection systems into microfluidic platforms, so relatively recent studies can be found ranging from confocal Raman spectroscopy [21] to fluorescence imaging [22].

Microfluidic devices for colorimetric carbon dioxide measurement are still relatively underdeveloped and only a few examples are found in the literature [23-26]. A low-cost, compact analytical microsystem for carbon dioxide determination in wine and beer production processes have been developed using a hydrophobic polyvinylidene fluoride membrane, which allows the transfer of the carbon dioxide in the sample to a bromothymol blue pH-sensitive acceptor solution, where the absorbance is the analytical signal. The detection system consisted of an LED with an emission peak at 607 nm LED and a photodiode integrated on a printed circuit board [23], although the detection limits established are high for environmental applications. A fully developed autonomous microfluidic system for CO_2/pH measurements in sea water has been reported by Rérolle et al. [1]. It incorporated microfluidic technology, an LED as the light source, and a USB spectrophotometer as the detector. The pH-sensitive material employed in this case was thymol blue.

In another example, the sensor was based on a microfluidic platform and contained a cross-reactive array composed of a halochromic dye/quaternary ammonium ion pair electrostatically bonded to ion-exchange polymeric microbeads. The optical setup was based on LEDs and a photodiode. Sensor sensitivity to low concentrations of

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dissolved CO_2 was subject to improvement. Residence time inside the microfluidic device has not been optimised in this study [24]. The "PreSens pCO_2 mini system" was evaluated for its ability to monitor changing dissolved CO_2 concentrations inside a microfluidic device to be used in a microfluidic biological culture device. Two configurations were evaluated: a flow-through cell external to the microfluidic device and a sensor spot housed inside the device. Unfortunately, this system is poorly described, it does not contain usual specifications such as LOD [25]. A paper-based platform for the detection of dissolved CO_2 has been described in which dissolved ammonia is sensed at the same time. A scanner was used for the detection system and the images were processed in MATLAB®. This system is promising but needs improvements in the detection system in order to be used *in situ* [26]

In this work, we describe the development of a microfluidic device capable of measuring the concentration of dissolved CO_2 up to 425.6 ppm using an aqueous reagent containing the pH indicator thymol blue. To the best of our knowledge, this is the first microfluidic device for the determination of dissolved CO_2 that measures colour. The usual way to measure in colorimetry is the use of color spaces, the most used is RGB (red, green, blue) and the HSV color space (hue, saturation, value) is also well known [27]. The advantage of colour measurements versus absorbance measurements lies in the variety of colour coordinates that could be used as an analytical parameter (R, G, B; H, S, V, a quotient from any two from each set, etc.) as well as the simplicity of the detection system. These coordinates come from the colour spaces RGB and HSV.

Our progression is described, starting from optimisation of the sensing chemistry using bench-top instrumentation first with cuvettes, second with a flow-injection system, and finally integration in a microfluidic system

2. Materials and methods

2.1 Reagents and materials

Thymol blue sodium salt (TB), sodium hydroxide, and sodium hydrogencarbonate were sourced from Sigma–Aldrich Química S.A. (Spain). All aqueous solutions were made using reverse-osmosis type quality water (Milli-RO 12 plus Milli-Q station from Millipore, conductivity 18.2 MΩ·cm).

2.2. Reagent preparation

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The aqueous reagent mixture was prepared by adjusting the pH of an aqueous solution 0.25 mM of TB to 8.2 using NaOH in order to obtain the optimum pH for CO_2 measurement.

2.2 Spectrophotometric measurements

Spectroscopic measurements were performed on a Hewlett Packard diode array spectrophotometer (model 8453; Nortwalk, CT, US). Different concentrations of hydrogen carbonate solutions were mixed with the reagent mixture solution at a 1:1 ratio (V/V) in 1.5 mL cuvettes with a path length of 1 cm. The absorbance spectra were recorded at 350–650 nm. In order to test the stability of the reagent, the afore-mentioned measurement system was repeated over three months.

2.3 Flow injection analysis

In order to simulate real application scenarios, a flow injection analysis technique (FIA) was used after the spectrophotometric analysis and before the inclusion of the reagent in the microfluidic chip. This is an important step towards realising a fully automated platform that can simple, add reagent and perform optical analysis in an integrated manner.

This technique started as an alternative to obtain automated analysis systems with a fast response. In this methodology, continuous flow is bound with spectrophotometric detection [28, 29].

The flow analysis setup consisted of a Gilson Minipuls-2 (Gilson, Inc., Middleton, USA) four-channel peristaltic pump working at a constant flow rate and two variable-volume Rheodyne 5041 teflon rotary valves (one as an injection valve and the other as a selection valve). PTFE tubing (Omnifit, Cambridge, England) (0.8 mm i.d. and 1.6 mm o.d.) and various end-fittings and connectors of different diameters were used to construct the flow system.

In order to verify the feasibility of this system, the FIA was tested using the reagent mixture described in Section 2.2. The carrier was the reagent mixture and every 90 seconds approximately 0.1 mL of samples of different CO_2 concentration were injected into the flow system. The flow rate of the peristaltic pump was set at 6.0 mL/min.

2.4 Microfluidic Chip

2.4.1 Design and Fabrication

To achieve highly sensitive, on-chip absorption measurements for the determination of carbon dioxide, three microfluidic chips were fabricated (Fig. 1) using a method previously described by Cogan et al. [30]. PMMA (2.0 mm, Access Plastics, Ireland) was milled using a Datron CAT3D-M6 milling machine to produce sample and reagent inlet channels and a serpentine mixer to allow for complete mixing prior to detection. The channel layer was subsequently sealed using thermal bonding to attach a 2 mm PMMA capping layer. An optical detection cuvette was attached to this chip using pressure-sensitive adhesive. The optical cuvette was a flow cell-type design for continuous sample throughput consisting of a 25 mm $\times$ 3 mm $\times$ 3 mm channel with a mirrored channel roof and milled Teflon walls. All other channels have a dimension of 0.5 x 0.5 mm. The chip was paired with an in-house 3D-printed holder with an incorporated detection system consisting of a white LED model C503D-WAN-CCBEB151 (Cree, USA) and a high-resolution colour detector model S11059 (Hamamatsu Photonics, Japan). This is an optical digital device that codifies the measurement of the incident light in four words of 16 bits each, corresponding to red, green, blue, and infrared (R, G, B, and IR). The output data of the colour detector is transmitted through a two-wire bus to an Arduino Uno board (Arduino, Italy) connected to a PC via USB, from which the user can start the measurements and read the results.

The LED was positioned at 45 degrees to allow it to irradiate the sample and to reflect the beam to the colour detector (Fig. 1).

Figure 1

2.4.2 Microfluidic system characterisation

Two different methods of analysis were carried out to analyse the operation of the CO_2 analysis via the microfluidic chip. Initially, premixed solutions were pumped into the system (the same solutions tested using bench-top instrumentation) with a peristaltic pump (Gilson Minipuls-2) to optimize the response of the LED/colour detector. Prior to any measurements, the microfluidic chip and hence all the channels were washed with deionised water. The premixed solutions were inserted in the chip (Fig. 2) by taking both liquid intake channels from the same source (channels 1 and 2).

Both channels of the peristaltic pump were set at the same flow rate (0.6 ml/min) and both solutions were pumped for 3 min for each measurement, at which point the flow

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was stopped and colour data were recorded. Each measurement is obtained as a triple set of characteristic colour coordinates (R, G, and B), as explained above. From these coordinates, the Euclidean Colorimetric Distance (D) is defined as the difference between the initial colour coordinate and the final colour coordinate of the analysed sample [31].

$$D = \sqrt{(R-R_0)^2 + (G-G_0)^2 + (B-B_0)^2} \qquad \text{(eq. 1)}$$

where R_0, G_0, and B_0 are the colour coordinates corresponding to the measurement of a CO_2-free sample. Using Eq. 1, each colour measurement is quantified by a single value.

The average of this six-value dataset was calculated (D) and considered representative of the sample colour. All experiments were made in triplicate, except when stated otherwise, to check for experimental errors.

 Once the detection system was optimised using premixed solutions, the system's operation was tested by mixing the different solutions (reagent/sample) on-chip. In this case, the reagent was taken from solution source 1 and the solutions containing different concentrations of carbon dioxide were taken from source 2. This method ensured a 1:1 (v/v) ratio for solution mixing prior to measurement; the solutions were pumped for 3 minutes for each measurement (the same as for premixed solutions). Measurements were taken for one minute. In order to ensure the correct mixing of reagent/sample, the waste from each mixture was recovered in small cuvettes and the spectra recorded using the spectrophotometer.

This chip is designed with three inlet channels in case a third solution is needed. In this application, only two solutions are used, so this third channel is blocked.

Figure 2

3. Results and Discussion

3.1 *Characterisation of the selected reagent mixture*

The optimum carbon dioxide reagent formulation was investigated by comparative analysis of different concentrations of TB and NaOH. Figure 3 presents an image from 21.3 ppm to 425.6 ppm of CO_2 showing the different colours obtained at the CO_2 concentration studied. Figure 4A shows the spectra of the dye. Visually, there

appear to be two colours (blue and yellow) whose peaks correspond to 440 nm and 595 nm. The highest wavelength was selected, as it is the most intense.

Figure 4B shows the absorbance (595 nm) plotted as a function of CO_2 concentration, which appears to exhibit an exponential decay dependence.

Figure 3

Figure 4

In order to check the behaviour of the selected chemistry in a fluidic system, experiments were carried out using the FIA system described above. Figure 5A presents the results obtained using the FIA system to vary the CO_2 concentration from 0 to 425.6 ppm. Figure 5B gives the average absorbance versus CO_2 concentration. As can be seen, the same trend as in previous experiments (Section 3.1) is obtained over the range studied.

Figure 5

The results obtained using FIA are in concordance with those obtained using bench-top instrumentation, so the flow has not changed the behaviour of the chemistry or the detection method. However, the sample and reagent consumption are still too high and, moreover, the experimental set-up is not portable, limiting its use to laboratory measurements. Due to these drawbacks, the integration in a microfluidic chip is necessary.

3.2 Microfluidic Chip Analysis

3.2.1 Optimisation of detection system

Initially, system response was optimised by adjusting the LED intensity and integration time of the colour detector using premixed solutions. The optimization was carried out by measuring the maximum and minimum CO_2 concentrations; the integration time was set to a value of 100 ms in order to obtain the maximum difference in parameter D corresponding to the extreme concentrations. With this configuration of the colour detector, the LED current was set at the highest possible

value with the aim of increasing the light collected by the colour detector and therefore the full scale, but avoiding saturation of the output.

Figure 6 represents a calibration function obtained when different concentrations of carbon dioxide are pumped within the chip. As can be seen, a linear trend is obtained in the studied range.

Figure 6

Mixing of Solutions on Chip

The next step was mixing solutions on chip instead of premixing. Figure 7 shows a calibration function when the sample and reagent are mixed on-chip.

It appears that the trend is similar to that obtained in the previous experiment with premixed solutions. However, the sensitivity value (taken as the slope of the linear fit) is less than for the premixed solution experiment. The explanation may lie in some experimental conditions for this reaction because the mixing was correct.

Figure 7

From this calibration curve, specifications such as the resolution and limit of detection (LOD) can be obtained. The resolution is calculated by taking derivatives on both sides of the equation and approximating these derivatives to increments [5]

$$\Delta CO_2 = \frac{df(D)}{dD}\Delta D \qquad (2)$$

where f(D) is the fitting function and ΔD is the error or uncertainty in the determination of the colorimetric distance D. This resolution depends on the CO_2 concentration since ΔD is not constant. The average value in the studied range is 5.96 ppm. The LOD was obtained using the standard criteria: $LOD = y_b + 3s_b$, where y_b is the average blank signal and s_b is the standard deviation of the blank, which is determined using at least 10 replicas. The value obtained is 7.24 ppm, which is a significant improvement for the typical LOD found in the literature for these kinds of systems (83 ppm) [23].

The precision study, carried out at three different concentrations (21.3 ppm, 127.7 ppm, and 425.8 ppm), generated the following results: 10.30, 3.23, and 3.17, respectively, for 10 replicate measurements at each concentration.

Once the calibrations functions were established, different solutions were pumped in order to know the feasibility of this new system. Solutions of the following concentrations were pumped into the system: 42.6 ppm, 191.6 ppm and 425.6 ppm obtaining a concentration value for each of 46.8 ppm, 183.1 ppm and 425.8 ppm, respectively. Percentage relative errors are calculated (%RE) were 10%, 4.44% and 0.05% respectively.

The following table compares the present reported method with literature reported methods.

Table 1. Comparison between current work and literature reported methods

Detection/ Dye	Range (ppm)	LOD (ppm)	Precision (%)	Flow rate ($\mu L \cdot min^{-1}$)	Response Time	Application	Ref.
A/ BTB	83-10000	83	2	400	N/S	Bottled wine and beer	[23]
A/ CR, P	Up to 85.2	NS	NS	NS	Long response time but not specified	variety of applications but it has not been applied	[24]
A/NS	Two concentrations were studied $50 \cdot 10^{E4}$ - $10 \cdot 10^{E4}$	NS	40	30	10 min	Culture media	[25]
Colour (LED-Scanner) / MR, Zn-TPP, P, CR	14.7-147	14.7	NS	Paper-based	N/S but must be long because PCA is needed	Chemical and biological sensing but it has never been applied	[26]
Colour (LED-Colour detector) / TB	7.2- 425.6	7.2	21.3 ppm➜ 10.30 127.7 ppm➜ 3.23 425.8 ppm➜3.17	600	100 ms	Natural waters	Current work

A: Absorbance BTB: Bromothymol blue, CR: Cresol red, P: Pyranine, NS: Not specified, MR: Methyl red, TB: Thymol blue

The present work is the fastest and shows the lowest LOD, besides is the only portable system capable of measuring CO_2 based on colour measurements, which implies more reliable and simpler systems.

3.3 Reagent Stability

The reagent shelf life is a very important issue for any real application as it will influence the potential duration of field deployments. In order to assess the stability of the CO_2-reagent, calibrations were carried out for three months on a weekly basis. Reagents were prepared and stored in the dark at room temperature for the total duration of the analysis, and aliquots were taken each week for analysis.

The results are shown in Figure 8 in terms of the stability of CO_2 calibration plot. Each data point represents the average of all measurements carried out during the three months of the analysis. The standard deviation is represented as error bars, and

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an excellent fit (exponential decay) is depicted by the black solid line (R^2=0.9986). It can be observed in Figure 8 that there is no significant difference between the CO_2 calibration points obtained at different dates as the standard deviation in all cases is minimal.

Figure 8

Conclusions

A new microfluidic system for the determination of dissolved carbon dioxide has been designed, developed, and tested using a combination of thymol blue with sodium hydroxide. Experiments were carried out first using benchtop instrumentation and finally integrated in the microfluidic chip. The design of a microfluidic chip coupled with a white LED and a colour detector successfully formed the detection system. The colour shift is quantified by means of a Euclidean colour distance defined as the distance to the reference colour in a CO_2-free sample, which reduces the total colour variation to a single parameter.

Working conditions were optimised using premixed solutions and mixing on chip was carried out obtaining linear fitting curves. In addition, the stability of the selected reagent was studied, as it is a crucial variable for any real application. This mixture has been studied for three months so far and is still stable. The system has been tested up to 425.6 ppm of CO_2. An average resolution of 5.9 ppm and a LOD of 7.2 ppm have been obtained, making this system suitable for multiple applications such as hydrology, biogeochemistry, and ecology. The result is a portable and autonomous carbon dioxide measurement system that could be deployable in the future for different environmental and industrial applications. This versatile system has been used for CO_2 determination but could form the basis for any analyte determination just by the selection of the adequate colour coordinate and the LED used as light source.

Acknowledgements

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Figure Legends

Figure 1. A. Microfluidic chip (40 x 30 mm), detector channel with mirror (28 x 3 mm). B. Schematic of the detection system formed by a white LED and the colour detector.

Figure 2. Schematic of the microfluidic and pumping system used.

Figure 3. Captured image of prepared CO_2 solutions from 21.3 to 425.6 ppm (from left to right).

Figure 4. A) Absorbance spectra of the reagent mixture. B) Absorbance at 595 nm as a function of carbon dioxide concentration. The points are the mean of three measurements and the standard deviation is represented as error bars.

Figure 5. A) FIA response from 0 to 425.6 ppm of CO_2. B) Absorbance at 595 nm as a function of carbon dioxide concentration. The points are the mean of three measurements and the standard deviation is represented as error bars.

Figure 6. Calibration plot using pre-mixed solutions. The represented points are the mean of three measurements at 595 nm, and the standard deviation is represented as error bars. The calibration equation is D= 6.3 [CO_2] + 1231.5, R^2= 0.9872

Figure 7. Calibration on-chip. The points are the mean of three measurements at 595 nm, and the standard deviation is represented as error bars. The calibration equation is D= 2.8 [CO_2] + 902.8 , R^2= 0.9809

Figure 8. Stability of selected CO_2 reagent for a duration of three months. The points are the mean of three measurements, the standard deviation is represented as error bars, and the black solid line is a polynomial fit.
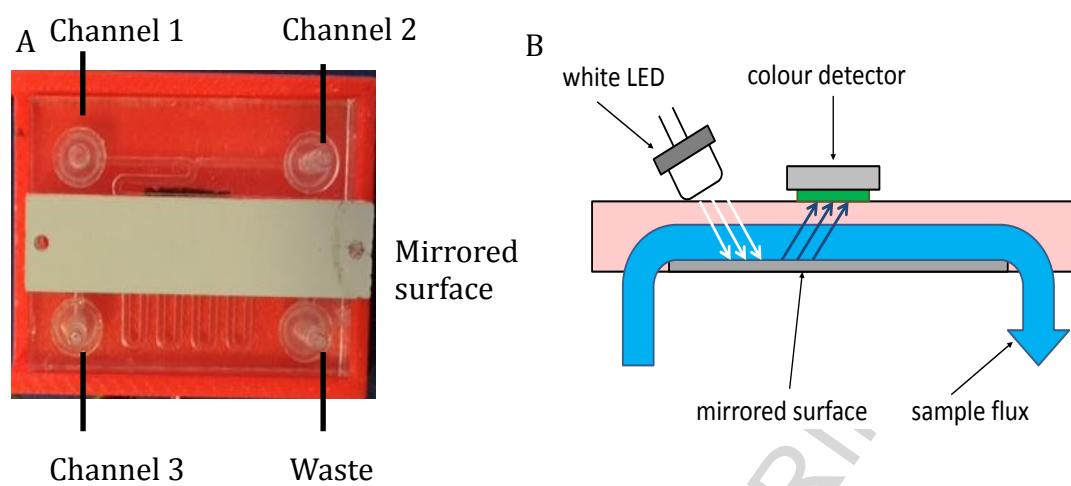
17

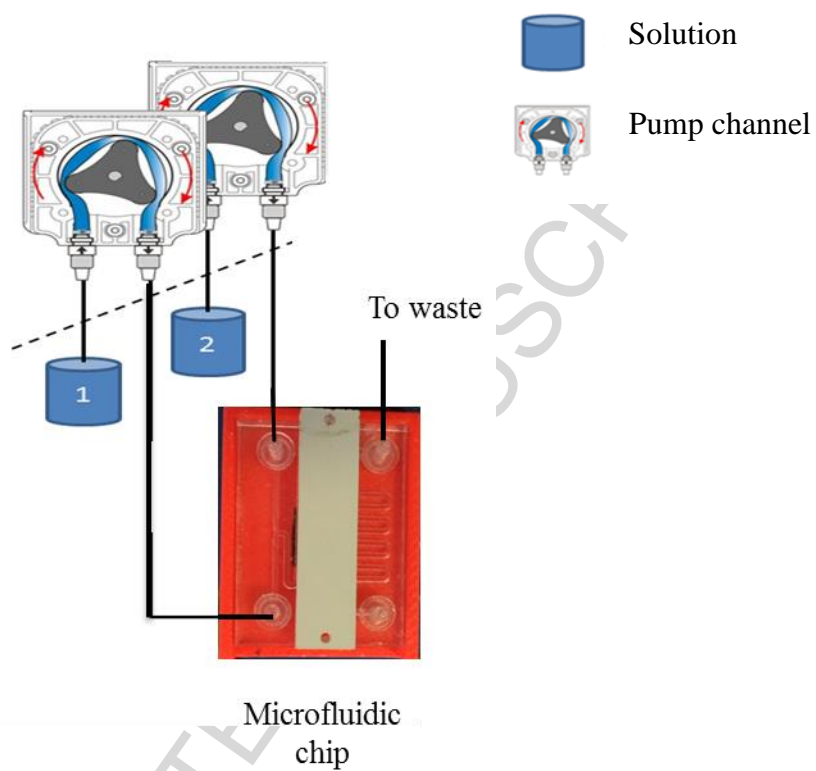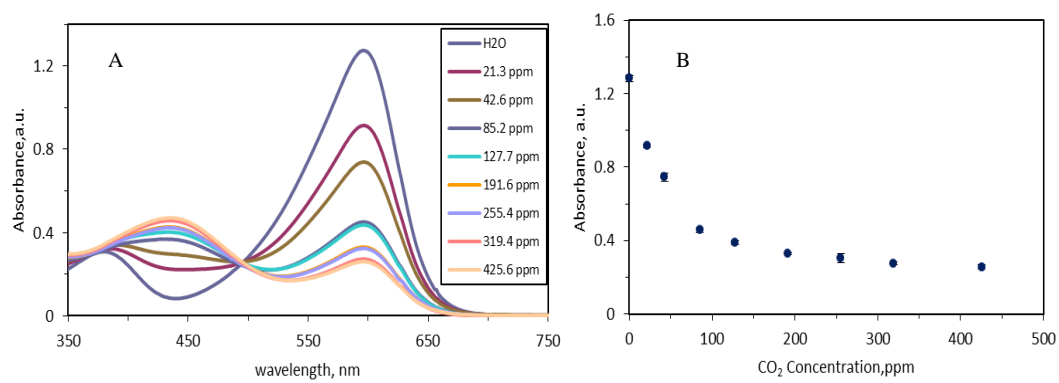A Channel 1   Channel 2

B

Channel 3   Waste

Mirrored surface

white LED   colour detector

mirrored surface   sample flux

Figure 1

Solution

Pump channel

To waste

Microfluidic chip

Figure 2

Figure 3

Figure 4

Figure 5

$$D = 6.3[CO_2] + 1231.5$$
$$R^2 = 0.9872$$

Figure 6

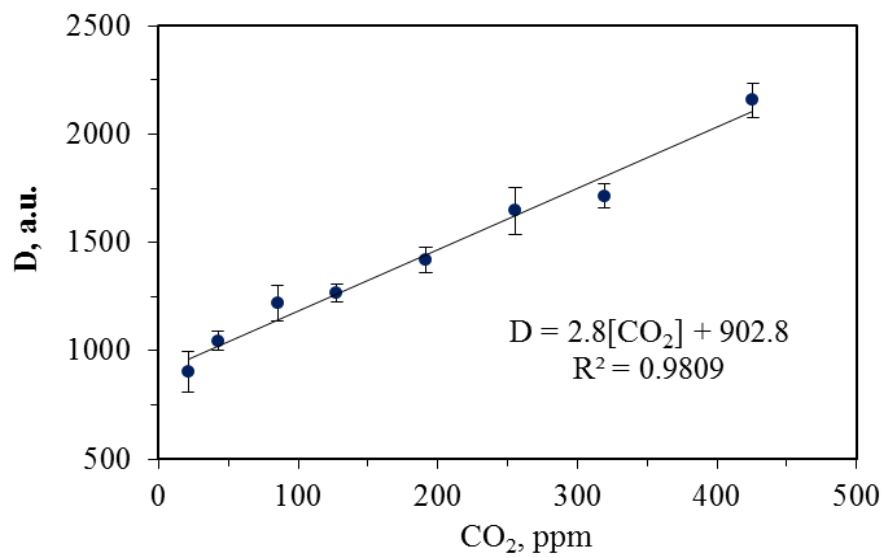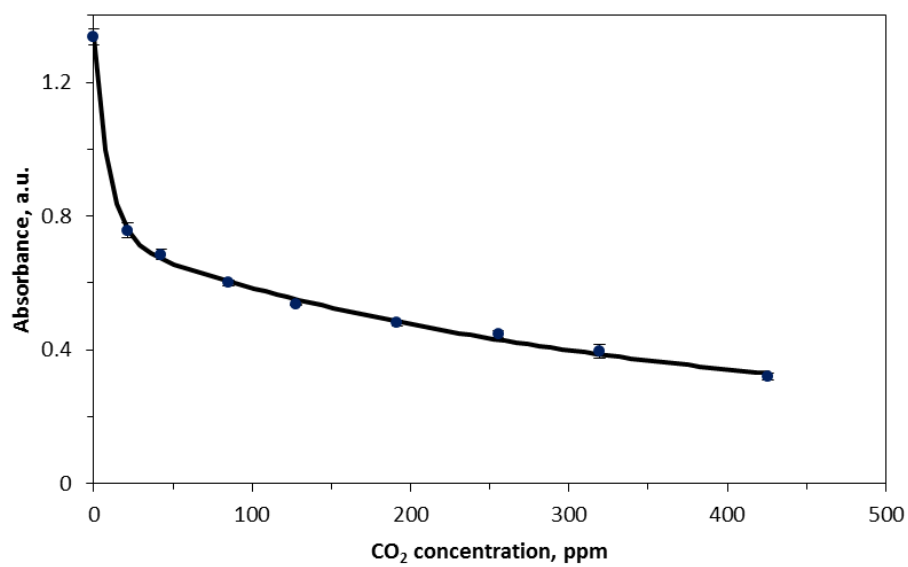$$D = 2.8[CO_2] + 902.8$$
$$R^2 = 0.9809$$
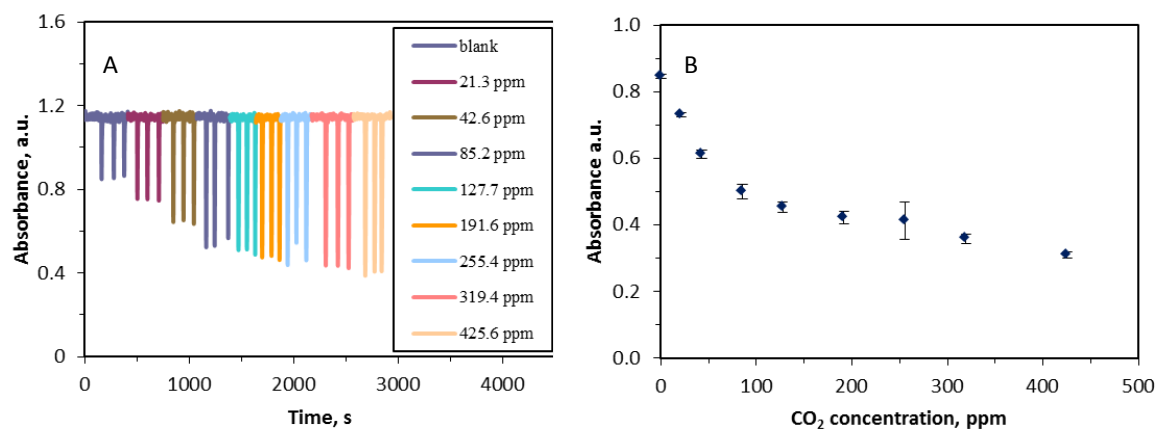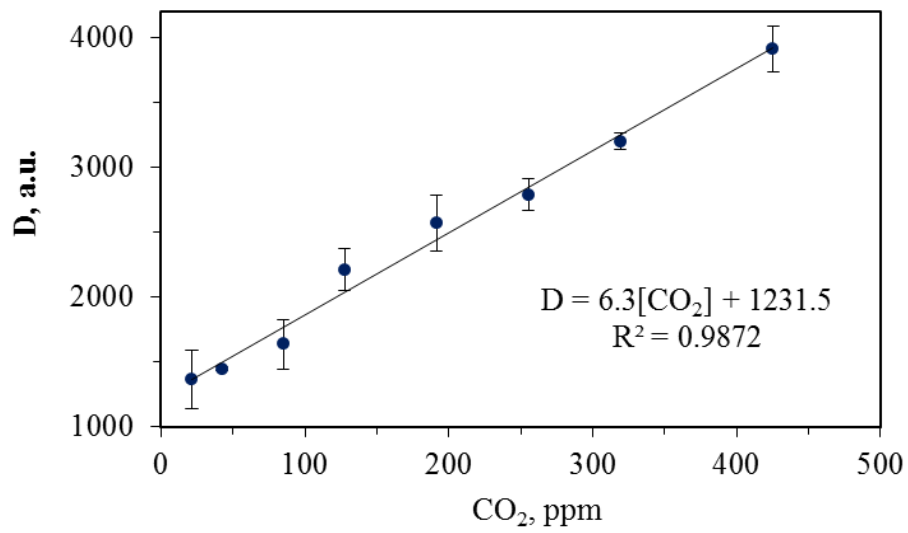
Figure 7

Figure 8

Highlights

- A microfluidic sensor for dissolved carbon dioxide determination in aqueous environments has been developed
- The design of a microfluidic chip coupled with a white LED and a color detector successfully formed the detection system
- This is the first microfluidic device for the determination of dissolved CO_2 that measures color
- The stability of the selected reagent has been studied for three months so far and is still stable.
- The result is a portable system for the determination of dissolved carbon dioxide from 7.2 ppm to 425.6ppm