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Low cost and compact analytical microsystem for carbon dioxide determination in production processes of wine and beer

Antonio Calvo-López, Oriol Ymbern, David Izquierdo, Julián Alonso-Chamarro

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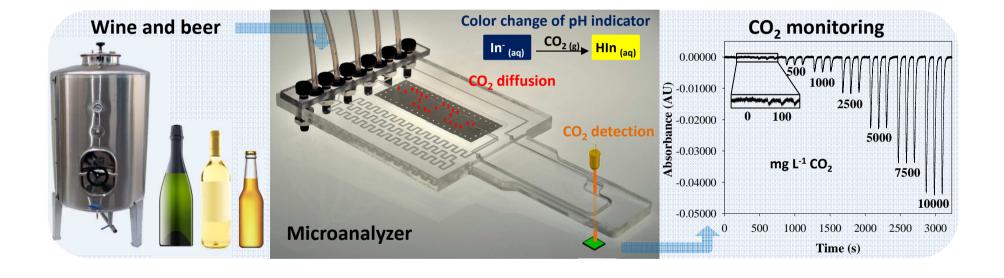
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- 1 Low cost and compact analytical microsystem for carbon dioxide
- 2 determination in production processes of wine and beer
- 3 Antonio Calvo-López^a, Oriol Ymbern^a, David Izquierdo^{b,c} and Julián Alonso-
- 4 Chamarro^{a,}*
- 5 ^aGroup of Sensors and Biosensors, Department of Chemistry, Autonomous University of
- 6 Barcelona, Edifici Cn, 08193 Barcelona, Spain
- 7 bCentro Universitario de la Defensa (CUD), Academia General Militar, Carretera de Huesca s/n,
- 8 Zaragoza, 50090, Spain
- 9 ^cGrupo de Tecnologías Fotónicas (GTF), Aragon Institute of Engineering Research (I3A),
- 10 Universidad de Zaragoza, Mariano Esquillor ed. I+D+i, Zaragoza, 50018, Spain
- *Corresponding author. E-mail address: <u>Julian.Alonso@uab.es</u>; Tel: +34935812149

12 Abstract

The design, construction and evaluation of a low cost, cyclic olefin copolymer (COC)-based 13 continuous flow microanalyzer, with optical detection, to monitor carbon dioxide in bottled 14 wines and beers as well as in fermentation processes, is presented. The microsystem, 15 constructed by computer numerically controlled (CNC) micromilling and using a multilayer 16 approach, integrates microfluidics, gas-diffusion module and an optical flow-cell in a single 17 polymeric substrate. Its size is slightly bigger than a credit card, exactly 45 x 60 x 4 mm in the 18 microfluidic and diffusion module zone and 22.5 x 40 x 3 mm in the flow-cell zone. The gas-19 diffusion module is based on a hydrophobic polyvinylidene fluoride (PVDF) membrane, which 20 21 allows the transfer of the carbon dioxide present in the sample to a bromothymol blue (BTB) 22 pH-sensitive acceptor solution, where the color change is measured optically. The detection system consisted of a LED with an emission peak at 607 nm and a photodiode integrated in a 23 24 printed circuit board (PCB). The obtained analytical features after the optimization of the microfluidic platform and hydrodynamic variables are a linear range from 255 to 10,000 mg L⁻¹ 25 of CO₂ and a detection limit of 83 mg L⁻¹ with a sampling rate of 30 samples h⁻¹. 26

- **Keywords:** Lab on a chip, Cyclic Olefin Co-polymer, Miniaturization, Gas-diffusion, Carbon
- 28 dioxide, Optical detection.

1. Introduction

29

30 The amount of carbon dioxide (CO₂) is an important parameter in the monitoring of 31 fermentation process in wines and beers [1] and it is also a factor that affects their organoleptic properties [2,3]. For these reasons, CO₂ is routinely controlled in the production processes of 32 33 these beverages in wineries and breweries. 34 There were traditionally three different official methods to determine CO₂ in these samples: the manometric, the enzymatic and the titrimetric, being the last one selected as first option due to 35 its advantages over the others methods [4,5]. This procedure consisted in a titration with sulfuric 36 acid between pH 8.6 and 4.0 of the alkalinized CO₂, using a pH electrode. Despite of its 37 38 advantages, the titrimetric method shows some difficulties. In order to overcome them, direct measurements of dissolved carbon dioxide, especially those based on electrochemical methods, 39 have also been proposed [6]. However, those methods based on Severinghaus principle show a 40 long response time and sometimes low reproducibility and, in general, they are often expensive, 41 42 not miniaturized and not easily automatable. As another alternative, automated systems based 43 on continuous flow methodologies with spectrophotometric detection and with gas-diffusion 44 separation step, have been used [7-9]. In this way, different quality parameters such as the analysis time, the sample and reagent consumption and the reproducibility of the measures 45 46 without the need of skilled laboratory personal, were improved. In these systems, the process to determine CO₂ usually starts when the sample is injected into a carrier solution which is mixed 47 with an acidic donor solution. At that moment, all the inorganic carbon species are converted to 48 CO₂, which diffuses through a gas-diffusion membrane. Then, a pH-sensitive acceptor stream 49 50 collects the CO₂ causing a change in the pH which is detected by a change in the absorbance of the acid-base indicator. Nevertheless, these systems present a narrow working range that 51 difficult the determination of CO₂ in beers (about 5000 mg L⁻¹) and wines (from about 1000 mg 52

53	L ⁻¹ in slightly sparkling up to 12000 mg L ⁻¹ in very highly sparkling wines) [1,8]. In addition,
54	sample and reagent consumption are still too high and the size of the experimental set-up makes
55	them not portable, limiting their use in both discrete in-situ sample analysis and on-line
56	monitoring of fermentation process.
57	To overcome these drawbacks, the miniaturization of analytical systems can be applied to obtain
58	the so-called micro Total Analysis Systems (μTAS) [10,11]. Among all the different materials
59	that have been used to fabricate these miniaturized analyzers, such as glass, silicon or ceramics
60	[12], polymers offer great advantages over the others. They allow a fast and easy fabrication
61	using inexpensive facilities providing low cost, small and compact microanalyzers. In addition,
62	depending on the polymer employed, they have a good transparency in the UV-Vis range, good
63	mechanical resistance, high chemical inertia against most acids and alkalis and high
64	biocompatibility [13-16]. Furthermore, polymers technology allows the fabrication of
65	microsystems using a multilayer approach in order to obtain tridimensional structures through
66	the use of adhesives between layers in the bonding process. However, when adhesives are used,
67	the obstruction of the microchannels can occur and there may be leaking of liquids or
68	delamination between layers. To overcome these drawbacks, cyclic olefin co-polimer (COC)
69	arises to provide a thermolamination process using the same material substrate as a sealing
70	agent. Thus, taking advantage from the different glass transition temperatures in which COC
71	can be formulated, a hermetic bonding of tridimensional structures can be achieved through the
72	interlink of polymer chains on the surfaces of the layers when they are put together under a
73	certain pressure and temperature [17, 18]. This technology allows also the monolithic
74	integration of other elements such as hydrophobic/hydrophilic polymeric membranes,
75	conductive paths or electrodes into the multilayer COC devices, which permits to integrate
76	different steps of the analytical procedure such as sample dosage, sample pretreatment and
77	separation, mixing, reaction, detection, among others [13,19, 20].
78	Finally, the advances in optoelectronics, which have let the appearance of new small light
79	sources such as light-emitting diodes (LEDs) [21-25] and small detectors such as photodiodes

80	[21-24], allow the possibility to reduce the size of the experimental set-up for optical				
81	measurements compared to the conventional equipments, making them easily miniaturized				
82	[18].				
83	Herein, we propose a low-cost, COC-based microsystem with a gas-diffusion step integrated for				
84	the spectrophotometric detection of CO ₂ using bromothymol blue (BTB) as pH-sensitive				
85	acceptor solution. The microanalyzer integrates microfluidics, gas-diffusion separation step and				
86	an optical detection cell. These features, joined to the simplicity of the detection setup, make the				
87	entire system easily portable, with a reduced consumption of sample and reagents and a wider				
88	linear working range. To demonstrate its applicability, different wine and beer samples have				
89	been analyzed.				
90	2. Experimental				
91	2.1. Reagents and materials				
92	The microanalyzer was fabricated with plaques and foils of COC purchased from Topas				
93	Advanced Polymers (Florence, KY, USA) in different grades and thicknesses: Topas 5013				
94	plaques of 500 μm and 1 mm thickness, and Topas 8007 foils of 25 μm and 50 μm thickness.				
95	A hydrophobic membrane made of polyvinylidene fluoride (PVDF) with 150 μm thickness and				
96	$0.45 \mu m$ pore diameter (Millipore) was used to separate the analyte from its sample matrix by				
97	gas diffusion.				
98	All reagents employed in this work were of analytical grade. All solutions were prepared in				
99	Milli-Q water. Carbon dioxide standard solutions were prepared daily by successive dilutions of				
100	a 50,000 mg $L^{\text{-}1}$ stock of Na ₂ CO ₃ (Panreac) and degassed. As a donor solution, a 0.2M H ₂ SO ₄				
101	acidic solution (Sigma-Aldrich), containing 60 mg L ⁻¹ of H ₂ O ₂ (Panreac) in order to avoid the				
102	SO ₂ interference by oxidizing it to sulfate [7], was used to convert all inorganic carbon species				
103	into carbon dioxide. As an acceptor solution, a 12 mM NaH ₂ PO ₄ /Na ₂ HPO ₄ (Panreac) buffer				
104	solution (pH 7.6), containing 0.06 mM of BTB (Sigma Aldrich), was used.				

2.2. Fabrication of the microanalyzer

105

106	The fabrication process of COC-based microsystems used, which is based in the lamination of
107	different COC layers with different glass transition temperatures (Tg), is described in detail
108	elsewhere [19,20]. Topas 6013 plaques with Tg=130 °C were used as fluidic structural layers for
109	the mechanization of all motifs of the design (microchannels and flow cell) and Topas 8007
110	foils with Tg=75 °C were used between them as sealing layers. The fabrication process consists
111	of four main steps: prototype design, fluidic motifs machining, integration of additional non-
112	COC elements (such as electrodes and gas-diffusion membrane) and final lamination. The
113	prototype design was performed with a CAD software (Figure 1) taking into account previous
114	experimental results obtained by our research group and described elsewhere [20]. The
115	microsystem was constructed using five structural layers that, once overlapped, provide the
116	three-dimensional structure required for this application (Figure 1A). The dimensions of the
117	microanalyzer were 45 x 60 x 4 mm in the microfluidic and diffusion module zone and 22.5 x
118	40 x 3 mm in the flow-cell zone which is inserted into the detection system based on a lock-and-
119	key concept [18] described in the 2.3 Experimental setup section. The microfluidics inside the
120	microsystem included three liquid inlets (Figure 3). Two of them converge in a T-shape
121	confluence point. At this point the carrier solution, where the sample is injected, and the H_2SO_4
122	solution are mixed to obtain carbon dioxide from all inorganic carbon species present in the
123	sample following the next chemical reactions: $CO_3^{2-}_{(aq)} + H^+ \leftrightarrows HCO_3^{(aq)}$, $HCO_3^{(aq)} + H^+ \leftrightarrows HCO_3^{(aq)}$
124	$H_2CO_{3(aq)}$, $H_2CO_{3(aq)} \leftrightarrows CO_{2(aq)} + H_2O$, $CO_{2(aq)} \leftrightarrows CO_{2(g)}$. The mixed stream is carried to the
125	diffusion membrane unit where, along a meander microchannel, the carbon dioxide diffuses
126	through the PVDF membrane towards the acceptor solution. The BTB pH-indicator acceptor
127	stream sets to pH 7.6 with 12 mM phosphate buffer collects the carbon dioxide diffused
128	changing its absorbance (from blue color to yellow color at wavelength of 607 nm) due to the
129	pH modification induced. Finally, both the donor and the acceptor solutions are carried to the
130	corresponding waste outlets.
131	All fluidic motifs (holes and channels) were machined onto the polymeric plaques by means of
132	a computer numerically controlled (CNC) micromilling machine (Protomat C100/HF, LPKF,

133	Spain). The dimensions of the microchannels throughout the microsystem were 0.4 mm wide			
134	and 0.3 mm height except in the zone where the diffusion process takes place. In order to			
135	maximize the contact area between the donor and acceptor solutions with the diffusion			
136	membrane, the dimensions of the channels in both cases were 1 mm wide and 0.1 mm height.			
137	The diameter of the detection flow-cell was 4.5 mm with an optical path-length of 1 mm. The			
138	total microsystem dead volume was 200 μ L. In order to increase the lifetime of the gas-			
139	diffusion membrane and to prevent its breakage, a non-coincident inlet streams configuration, in			
140	the gas diffusion unit, was used [20]. Thus, the stress on the membrane caused by the incidence			
141	of the two streams at the same point was avoided.			
142	The integration of the PVDF membrane was made as follows. It was cut, washed with deionized			
143	water, dried and placed properly with its more hydrophobic face in contact with the donor			
144	solution [20]. As it can be seen (Figure 1B), some holes were made on the external contour of			
145	the PVDF membrane to favor the penetration of the sealing foil (Topas 8007) during the			
146	lamination process in order to prevent liquid leakage between layers. In order to avoid the			
147	flattening of the membrane and the layers deformation during the thermo-compression process			
148	as a result of the incorporation of the membrane, an extra 50 μm thick Topas 8007 sealing foil			
149	with a machined hole with the dimension of the membrane area is placed between the two			
150	machined COC layers [19, 20].			
151	COC layers alignment and final lamination was performed in a thermo-compression press			
152	(Francisco Camps, Granollers, Spain) at 102 °C and 4 atm using an aluminum support with 4			
153	fiducial alignment pins. Therefore, the whole microsystem acts as a monolithic substrate with			
154	the different layers perfectly bonded between them. Finally, fluidic connectors were fixed onto			
155	the polymer inlet/outlet ports with a holder and screws (Figure 1B).			
156	2.3. Experimental setup			
157	The detection system developed previously by our research group has been described elsewhere			

[18,24]. It consists in a compact and robust optical reader for microfluidic platforms which is

158

159	composed by a structure with a Printed Circuit Board (PCB) for the optical detection electronics
160	and an insertion port, designed with a lock and key concept, to allow a perfect alignment
161	between the light source, the measurement flow-cell and the detector (Figure 2). The PCB
162	structure integrates as a light source a Light Emitting Diode (LED) with an emission peak
163	centered at 607 nm (Kingbright, Taipei, Taiwan) and as a detector, a PIN Hamamatsu S1337-
164	66BR with 33 mm ² of active area photodiode. The signal generated was acquired by means of a
165	Data Acquisition Card (DAQ) NI USB-6211 from National Instruments (Austin, Texas, US),
166	which transfers the signal from the PCB, via USB interface, to a personal computer, where it is
167	processed using a digital lock-in amplifier. The data collection rate was 10 s ⁻¹ . The use of lock-
168	in amplification allowed to increase the signal-to noise ratio and to work in ambient light
169	conditions avoiding thus the effect of stray light on the measures [18,24].
170	On the other hand, the continuous flow system setup is shown in Figure 3. It consists of an
171	external peristaltic pump (Minipuls 3, Gilson, Wisconsin, US) using Tygon® tubing (Ismatec,
172	Wertheim, Germany) with 1.14 mm internal diameter and a six-way injection valve (Hamilton
173	MVP, Reno, US). Teflon tubing (Scharlab, S. L., Cambridge, England) of 0.8 mm internal
174	diameter was used to connect the external elements to the microsystem.
175	3. Results and discussion
176	3.1. Design and optimization of the analytical microsystem
177	The main goal of this work is the development of a low cost, simple, selective and robust
178	microanalyzer for the determination of carbon dioxide in bottled wine and beer and in their
179	fermentation processes at breweries or wineries, which monolithically integrates the
180	microfluidic platform, the gas diffusion module and the detection flow-cell. This microsystem
181	has to meet all the requirements for the proposed application, such as easy automation, small
182	size, limited consumption of reagents and a wide working range.
183	In a previous work [20], the design of the diffusion module was optimized in order to enhance
184	the robustness of the separation process and to increase the lifetime of the PVDF diffusion
185	membrane keeping suitable analytical features such as sensitivity, baseline stability and

186	detection limit. In this work, variables such as the flow mode (concurrent or countercurrent) and
187	the flow rate ratio between the acceptor and donor solutions inside the diffusion module were
188	tested, concluding that the same configuration previously optimized (countercurrent flow mode
189	and 2:1 donor: acceptor ratio) is also the better choice for the present work. Additionally, the
190	microfluidic structures and the hydrodynamic variables has been designed and optimized
191	respectively to minimize the sample and regents consumption.
192	The shape of the flow-cell has also been redesigned and optimized introducing smooth-contour
193	geometries, without any sharp corners, and taking into account the Venturi effect and
194	Bernoulli's principle [26]. Thus, the formation and retention of bubbles was minimized inside
195	the flow-cell. Despite of this, if eventually bubbles appear, their evacuation is favored due to the
196	pressure-velocity difference of the fluid between the flow-cell and the outlet stream (Figure
197	1Bd). For this reason, noisy and transient signals and baseline drifts related with the presence of
198	bubbles in the flow-cell were avoided, increasing the overall robustness of the microsystem.
199	In addition, the shape of the microsystem was designed so that it can be inserted into a detection
200	system based on the lock and key concept (Figure 2), allowing a perfect and reversible
201	alignment between the LED, the detection flow-cell and the photodiode and thus, obtaining a
202	robust and compact system, as demonstrated in a previous work [18].
203	The influence of chemical variables (buffered acceptor solution, acidic donor solution and
204	potential interfering compounds) and hydrodynamic parameters (flow rate and sample injection
205	volume) on the analytical features has also been evaluated using an optimization of each
206	variable individually in order to achieve a compromise between the sensitivity of the analytical
207	measurements, the baseline signal stability, the linear working range, reagent and sample
208	consumption and the sample throughput.
209	According to the bibliography, bromocresol purple [9], phenol red [27] and bromothymol blue
210	(BTB) [7,19,27-29] have been used as pH-sensitive indicators dissolved in an acceptor stream to
211	determine carbon dioxide in real samples. In this work, BTB dissolved in a phosphate buffer
212	solution was selected because it showed a higher working range [29]. The BTB acceptor

solution was tested at concentrations ranging from 0.03 to 0.08 mM, the phosphate buffer
strength was evaluated from 0.1 to 12 mM and the pH of the acceptor stream was evaluated
from 7 to 8. The optimal results were obtained using an acceptor solution of BTB 0.06 mM at
pH 7.6 with phosphate buffer strength of 12 mM. Using this buffered acceptor solution, the
peak height caused by a given CO ₂ concentration diminishes compared to that obtained with a
non-buffered acceptor solution. This fact means that the higher the buffer strength, the lower the
peak height for each CO ₂ concentration and the lower the sensitivity, but at the same time it
allows enlarging the operative working range [19,28,29]. Taking into account that the aim of the
present work is to be able to determine a range of relatively high carbon dioxide concentrations
in wines and beers, a very low limit of detection and high sensitivity are not required. In
addition, using the buffered acceptor solution, the changes in the pH caused by the absorption of
atmospheric carbon dioxide are negligible. Nevertheless, in other applications when very low
concentrations of CO ₂ have to be determined, non-buffered acceptor solution should be used. In
these cases, the absorption of atmospheric carbon dioxide would be a serious problem which
can be minimized introducing cartridges of soda lime at the air inlet of the bottles of reagents to
completely remove the atmospheric CO ₂ [30].
On the other hand, hydrochloric and sulfuric acids were evaluated as donor acidic solutions
without obtaining any significant differences between them in terms of peak height. However,
sulfuric acid was selected due to that hydrochloric acid can diffuse through the PVDF
membrane, since it has a higher vapor pressure, and can change the pH of the acceptor solution.
The concentration of H ₂ SO ₄ donor stream was varied from 0.2 to 0.6 M. The optimal results
were obtained with a donor solution of H ₂ SO ₄ 0.2 M.
Taking into account potential interfering compounds that can be found in these samples, sulfite
ion is the only one that has to be taken into account because it is the only chemical compound
present in wine samples able to diffuse in an acidic medium, as SO ₂ , through the PVDF
membrane [7]. Its concentration in wine can be up to 300 mg L ⁻¹ SO ₂ . For this reason H ₂ O ₂ is
added to the donor stream in order to assure the total oxidation of SO_2 to SO_4^{2-} which cannot

240	diffuse through the membrane. The concentration of hydrogen peroxide used was optimized				
241	elsewhere [7], being 60 mg L^{-1} . Therefore, the final composition of the donor stream was 0.2 M				
242	of H ₂ SO ₄ with 60 mg L ⁻¹ of H ₂ O ₂ . Despite of this, due to SO ₂ usually is in less proportion than				
243	CO_2 in wine, $\mathrm{H}_2\mathrm{O}_2$ could be suppressed without obtaining significant differences in the results.				
244	Regarding the hydrodynamic parameters, the flow rate of the carrier, the donor and the acceptor				
245	streams were varied from 100 to 500 $\mu l \ min^{1}$ and the sample injection volume was varied from				
246	25 to 580 μ L. A flow rate of 400 μ l min ⁻¹ for carrier, donor and acceptor streams and a sample				
247	injection volume of 25 μL were considered the optimal parameters.				
248	3.2. Analytical performance				
249	Analytical characterization of the microsystem was carried out by successive calibrations				
250	obtained using different standard solutions of Na ₂ CO ₃ from 100 to 10,000 mg L ⁻¹ . Figure 4				
251	shows the recorded signal for one calibration. The obtained equation ($n = 8$ and 95%				
252	confidence) was Abs=-4.45·10 ⁻⁶ ($\pm 2 \cdot 10^{-8}$) [CO ₂] + 0 ($\pm 1 \cdot 10^{-4}$) with r^2 =0.9999. The detection				
253	limit, calculated as three times the standard deviation of the blank, was 83 mg L ⁻¹ . The linear				
254	working range corresponds from 255 to $10,000~{\rm mg}~{\rm L}^{-1}$. Repeatability studies were performed by				
255	successive injections of 2,500 and 5,000 mg L ⁻¹ Na ₂ CO ₃ standard solution. Relative standard				
256	deviation ($n = 6,95\%$ confidence) of the signals were lower than 2% in both cases.				
257	Reproducibility of the results obtained in different days using different reagent solutions was				
258	also determined. Mean slope of 4.38·10 ⁻⁶ with RSD value lower than 2% was achieved, thus				
259	demonstrating the reproducible inter-day validation of the microsystem. These results showed				
260	the robustness and the reliability of the whole experimental setup. A sampling rate of 30				
261	samples h ⁻¹ was obtained with the optimized experimental conditions.				
262	3.3. Real samples analysis				
263	Real samples of wines and beers from different origin were analyzed to validate the proposed				
264	microsystem. In order to cover a wide range of carbon dioxide concentrations, slightly sparkling				

265 wines, sparkling wines and beers were analyzed. With the aim of establish an accurate 266 comparison between the results obtained by both methods, before the analysis of the samples, 267 and following the procedure of the reference method [4,5], the bottles were kept at 4 °C, they 268 were opened and their content was immediately treated with 30 mL of 10 M NaOH per liter of 269 sample in order to fix the concentration of CO₂ and avoid its loss. Then the mixtures were 270 allowed to reach room temperature in closed bottles without air before their determination. It is 271 noteworthy that the developed microsystem can analyze real sample without any pretreatment. 272 As it has been stated previously, NaOH was added to allow an accurate comparison between 273 both methods. In the case that NaOH is not used, the longer the bottle is open, the more loss of carbon dioxide there is, so this is an issue to take into account. In the same way, the 274 275 microsystem is able to determine CO₂ online in fermentation processes in breweries and 276 wineries. Due to the logistic difficulties, these kinds of samples were not determined. 277 The results obtained with the developed optical microsystem were validated by comparison with the ones obtained with the titrimetric reference method. This procedure, as have already 278 mentioned, consisted in a titration of alkalized CO₂ with sulfuric acid, between pH 8.6 and 4.0, 279 using a pH-meter. The obtained results are shown in Table 1. 280 281 As it can be seen, the results of the developed microsystem are not significantly different from the ones obtained with the reference method using the paired t-test ($t_{calc} = 1.595$; $t_{tab} = 2.365$; 282 t_{calc}<t_{tab}). This fact confirms that the proposed analytical microsystem is useful for the 283 284 determination of carbon dioxide in a wide range of real samples. Furthermore, the achieved 285 precision is better than the one obtained with the reference method and comparable with the 286 precision of other carbon dioxide analyzers reported previously.

4. Conclusions

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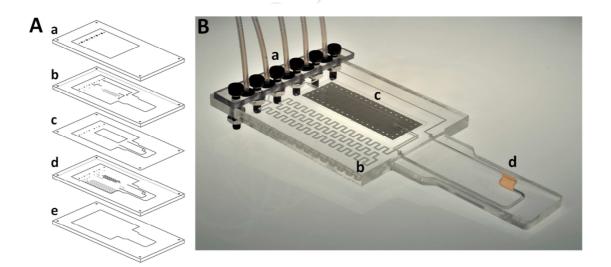
A low-cost, spectrophotometric microanalyzer for the determination of carbon dioxide in wines and beers, integrating a gas diffusion PVDF membrane, has been developed and applied to real samples. The microsystem is compact and robust and provides a fast response with lower

291	sample and reagent consumption, wider working range and higher degree of miniaturization				
292	than other carbon dioxide analyzers previously reported [1,7]. In addition, the use of a lock and				
293	key concept allows the use, as a detection system, of a miniaturized optical reader for				
294	microfluidic platforms where the microsystem can be inserted, achieving a perfect alignment				
295	between the LED, the detection flow-cell and the photodiode. This approach makes the whole				
296	system simple, robust and compact providing a very good precision and repeatability in the				
297	measurements performed. In addition, overall features obtained enable also the possibility to use				
298	it for on-line fermentation process monitoring.				
299	Acknowledgments				
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Figure 1. **A:** Design of the layers of the prototype. Layers a, b, d and e are Topas plaques of 1mm, layer c is Topas foil of 50 μm. Layers b and d have different patterns on both sides, in front, black color, and behind, grey color; **B:** Picture of the final device: **a)** Fluidic connections; **b)** Microfluidics; **c)** PVDF membrane; **d)** Flow-cell depicted in orange color.

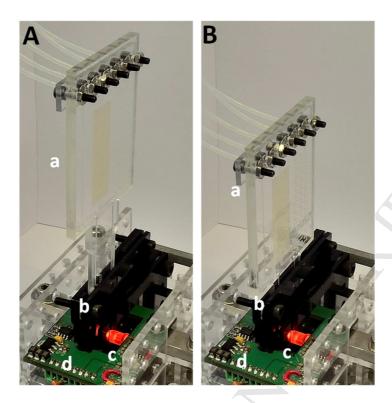


Figure 2. A and B: Picture of the microdevice outside and inside the detection system: **a)** microanalyzer; **b)** lock-and-key insertion port integrating a mask with a circular hole that only allows the pass of the light through the flow-cell; **c)** LED at 607 nm; **d)** PCB with the electronics associated to the control and acquisition of the signal.

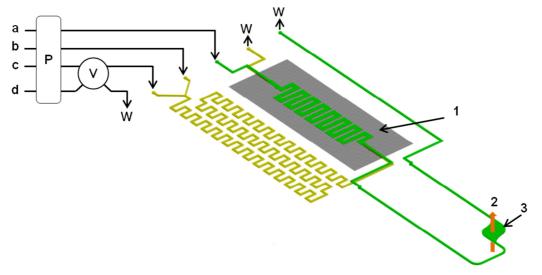


Figure 3. Schematic diagram of microfluidics, detection system and experimental set-up. a)

BTB buffered acceptor stream **b**) 0.2 M H₂SO₄ solution with 60 mg L⁻¹ H₂O₂; **c**) H₂O as carrier

solution. **d**) sample; **P**) peristaltic pump; **V**) six-way injection valve; **W**) waste outlets; **1**) PVDF membrane; **2**) direction of the light from the LED through the flow-cell; **3**) detection flow-cell; yellow color: microchannels under the PVDF membrane; green color: microchannels over the PVDF membrane.

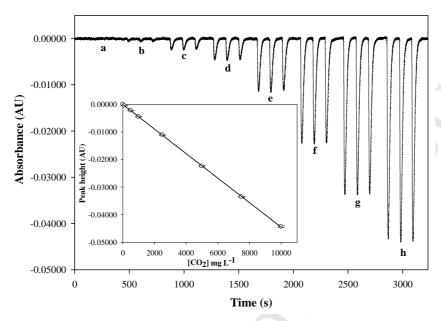


Figure 4. Signal recording and calibration curve for the microanalyzer using Na_2CO_3 standard solutions of 0 mg $L^{-1}(\mathbf{a})$, 100 mg $L^{-1}(\mathbf{b})$, 500 mg $L^{-1}(\mathbf{c})$, 1,000 mg $L^{-1}(\mathbf{d})$, 2,500 mg $L^{-1}(\mathbf{e})$, 5,000 mg $L^{-1}(\mathbf{f})$, 7,500 mg $L^{-1}(\mathbf{g})$ and 10,000 mg $L^{-1}(\mathbf{h})$.

398 Table 1

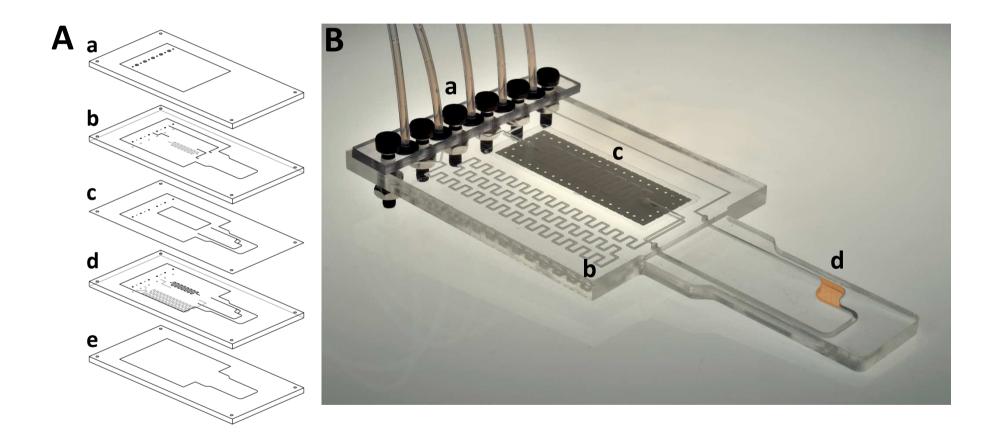
Mean concentration values in mg L⁻¹ (n=4, 95%) from the analysis of wine and beer samples using the proposed microsystem.

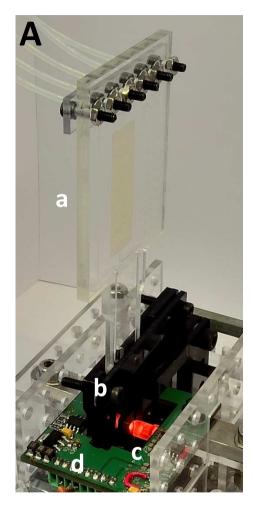
Sample	Microsystem	Reference method	% error
Spanish sparkling wine	6700 ± 100	7100 ± 320	6
Spanish white slightly sparkling wine	5000 ± 200	4800 ± 600	4
Spanish white slightly sparkling wine	4500 ± 200	4500 ± 700	0
Spanish white slightly	4100 ± 200	4300 ± 600	5

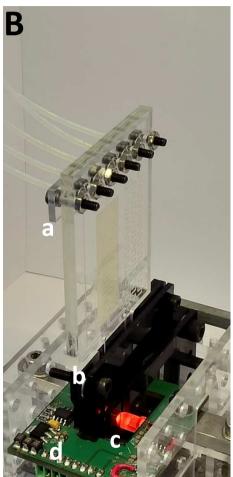
sparkling wine			
Spanish white slightly sparkling wine	3300 ± 100	3400 ± 300	3
Portuguese white slightly sparkling wine	2900 ± 200	3000 ± 300	3
Italian rosé slightly sparkling wine	5800 ± 200	6000 ± 300	3
Spanish beer	5400 ± 200	5400 ± 600	0

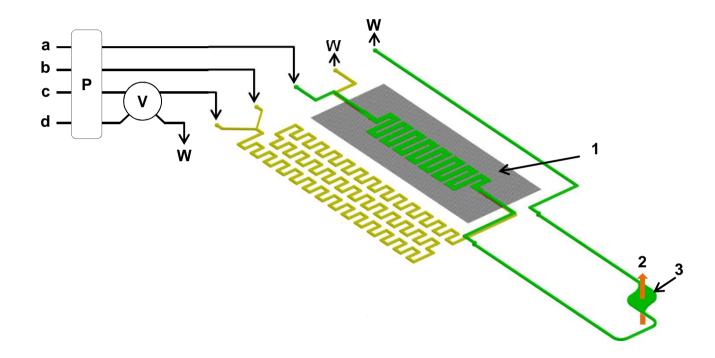
Table 1 $\label{eq:mean_concentration} \mbox{Mean concentration values in mg L^{-1} (n=4, 95\%) from the analysis of wine and beer samples using the proposed microsystem.}$

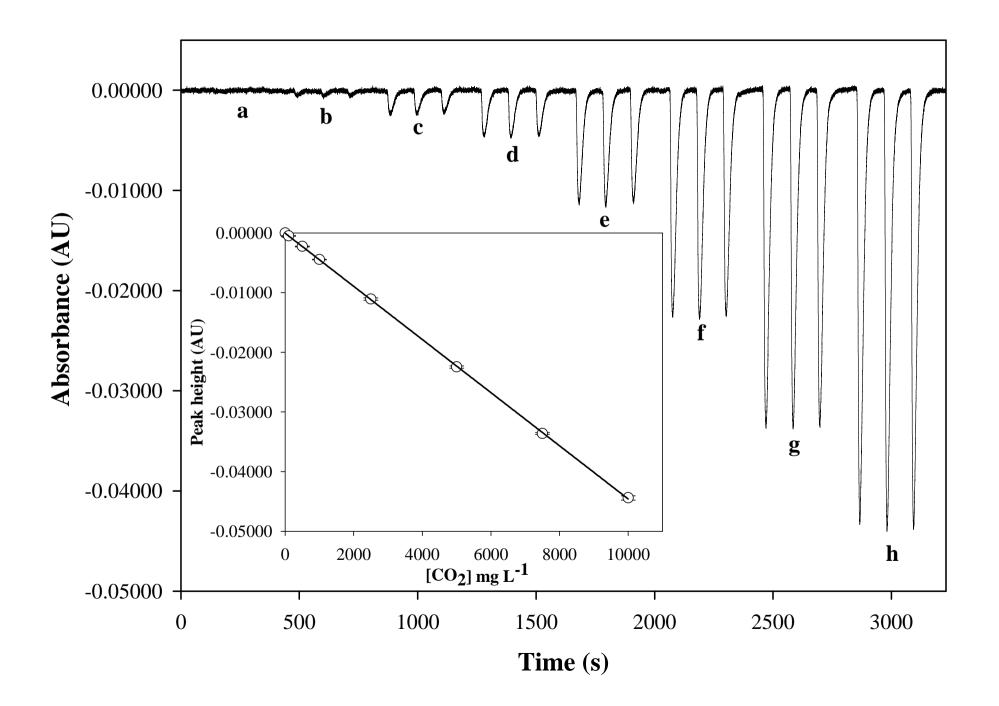
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Low cost and compact polymeric analytical microsystem.

Carbon dioxide determination using a gas diffusion step and optical detection

Real wine and beer samples were successfully analyzed

Automatic measurements to monitor production processes of wine and beer could be performed