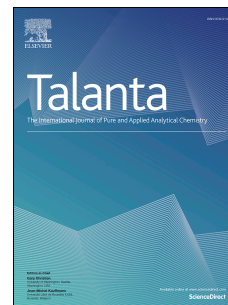# Journal Pre-proof

Electrochemical Micropipette-Tip for Low-Cost Environmental Applications: Determination of Anionic Surfactants through their Interaction with Methylene Blue

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Credit author statement

A.González-López: methodology, investigation, visualization, writing original draft; **E. Costa-Rama**: methodology, supervision, writing-review and editing; **M.T. Fernández-Abedul**: conceptualization, methodology, supervision, writing-review and editing.
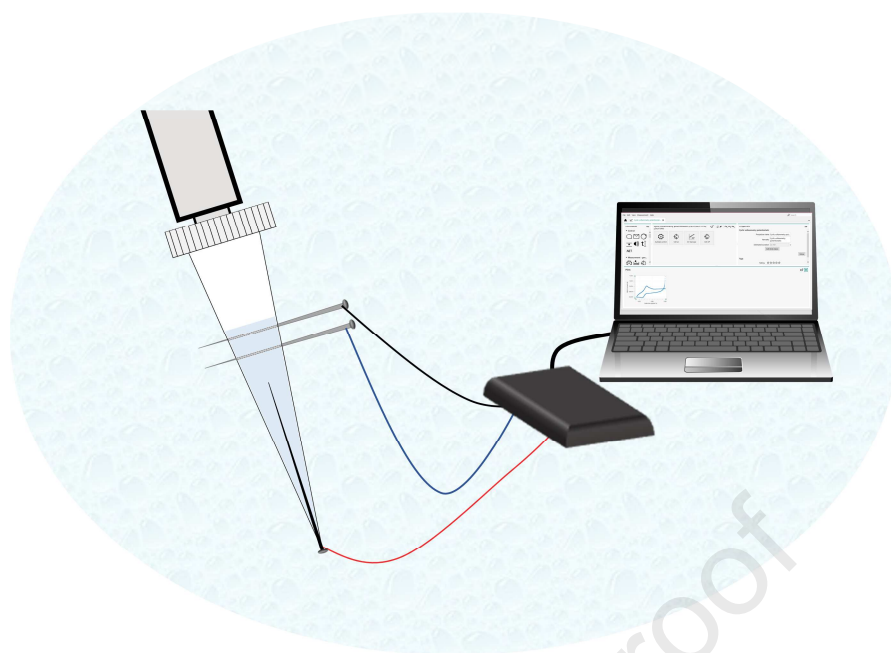
1 **Electrochemical Micropipette-Tip for Low-Cost**
2 **Environmental Applications: Determination of Anionic**
3 **Surfactants through their Interaction with Methylene**
4 **Blue**

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9 **Abstract**

10   Miniaturization is one of the main requirements of the design of portable devices
11 that allow in-field analysis. This is especially interesting in environmental monitoring,
12 where the time of the sample-to-result process could be decreased considerably by
13 approaching the analytical platforms to the sampling point. We employed traditional
14 mass-produced and low-cost elements (micropipette tips and pins) in an out-of-box
15 application to generate an innovative and cost-effective platform for analytical
16 purposes. We have designed simple and easy-to-use electrochemical cells inside
17 polypropylene micropipette tips with three stainless-steel pins acting as the working,
18 reference and counter electrodes of a potentiostatic system. The pin acting as working
19 electrode was previously coated with carbon ink, meanwhile the rest were used
20 unmodified. In this way, electrochemical measurements were done directly in-the-tip
21 using low volumes (µL) of sample. The devices showed good reproducibility, with a
22 relative standard deviation of 7% (n=5) for five different tip-based complete
23 electrochemical cells. As a proof-of-concept, its utility has been probed by the
24 determination of an anionic surfactant (sodium dodecyl sulphate, SDS) in water
25 through its interaction with methylene blue (MB). Two different alternatives were
26 presented: 1) electrochemical determination of the MB remaining in the aqueous phase
27 after extraction of the pair SDS-MB to an organic medium.

28 *Keywords: micropipette-tip electrochemical cell, stainless-steel analysis,*
29 *electrochemical analysis, water analysis, sodium dodecyl sulphate.*

30   **1. Introduction**

31   The design of miniaturized and low-cost sensing devices is an area of enormous
32 interest in the field of Analytical Chemistry. They are definitely required to move

33   (bio)chemical analysis from the bench to the field [1]. In fact, the combination of

34   wireless sensors with smartphones is producing platforms well suited to perform

35   decentralized analysis [2,3]. In this context, the electrochemical detection must be

36   pointed out since it is low cost and easily miniaturized and provide high selective and

37   sensitive methodologies [4].

38   In terms of cost-effectiveness, the use of common elements for out-of-box

39   applications is clearly increasing. They are already mass-produced items that can be

40   utilized for novel applications. In 2016, Whiteside's group introduced the use of

41   stainless-steel pins as electrodes in combination with thread for microfluidic

42   determination of lactate [5]. The modification of these metallic elements with carbon ink

43   allowed their use to determine glucose enzymatically, in static [6] and flow-based

44   systems [7]. Batch injection analysis has also been demonstrated for epinephrine

45   determination using pin-based cells [8]. Their low size enabled the design of arrays for

46   performing simultaneous measurements using a multichannel potentiostat [6].

47   Similarly, other mass-produced elements of common use such as staples have been

48   modified for use as electrodes in new electrochemical low-cost platforms [9].

49   Among the items that are widely employed in most (bio)chemical laboratories and

50   can be employed in out-of-box applications are micropipette tips. They can be used for

51   different purposes apart from dispensing liquids, their main function. These novel

52   applications include sample purification as in the case of tips containing a bed of

53   chromatography media for the detection of human chorionic gonadotropin (hCG) and

54   prostate-specific antigen (PSA) in urine samples [10] or selective extraction, with

55   integrated molecularly-imprinted monoliths, of the toxin berberine [11] or the pesticide

56   methomyl [12]. More recently, our research group designed an enzymatic

57   immunoassay where the inner surface of a micropipette tip was turned into a capture

58   zone for anti-tissue transglutaminase (IgA) [13], antigen of interest for the detection of

59   the celiac disease.

60   Moving further in this direction, in this work we add a new functionality to this

61   common and affordable consumable material, easily found in laboratories. With this

62   aim, we have integrated a very simple electrochemical cell. Already in 1991, a

63   micropipette tip was used as a platform for the fabrication of carbon fiber

64   ultramicroelectrodes [14]. Following this idea of using the tip as container, miniaturized

65   electrochemical devices were designed using micropipette tips combined with e.g.,

66   graphite composite [15] or epoxy resin [16]. In these examples, the micropipette tip

67   acted as the body of the working electrode, that had to be immersed in the sample

68   solution for performing measurements, as happens with e.g., a polymeric cylinder that

69 encloses carbon paste or a pencil lead. The other two electrodes (reference and
70 counter electrodes) had to be introduced in the solution as well, which could be done: i)
71 independently, as different elements, or ii) integrated in the outer part or the tip. Apart
72 from these approaches, the tips can be used, after sealing the bottom, as low-cost and
73 low-volume electrochemical cells. Following this, three small-size electrodes with a
74 pencil graphite working electrode were introduced in a tip-based cell [17]. However, in
75 all these cases the tip is modified and cannot be used in combination with a
76 micropipette for performing its original function: aspirating and dispensing solutions.
77 There is a unique report on the use of a micropipette-tip used as container of a
78 complete electrochemical cell [18] that retained this function. In this case, the sample
79 was aspirated and analyzed thanks to the introduction of three wire electrodes (with
80 gold-based working and reference electrodes) and a cotton wool filter (for medium
81 storage and sample purification) inside the tip, in a new lab-on-a-tip approach for
82 decentralized electroanalysis of Cu^{2+} in water.

83   In this work, we propose the use of a simpler and lower-cost device that employs
84 stainless-steel pins as electrodes and integrates them in the micropipette tip, which
85 becomes now multifunctional. A pin modified with carbon ink is used as working
86 electrode, and two more pins, unmodified, are used as reference and counter
87 electrodes. One important advantage is that the modification of pins with carbon ink
88 allows their use in many applications. Carbon materials, such as carbon paste/ink and
89 graphite, are widely used in electrochemistry because of their advantageous
90 properties: carbon is cheap and chemically inert and shows high surface area and
91 electrical conductivity, low background currents and a wide potential window [19,20].
92 The bottom of the tip has a diameter that allows the introduction of the pin covered with
93 a thick film of carbon, with the head of the pin acting as stopper. Following this new
94 design, the electrochemical cell can be fabricated in an extremely fast and reproducible
95 way employing inexpensive materials.

96   Thus, we present here a modified micropipette tip that act as an electrochemical
97 cell, very useful for in-field analysis. The combination of common (stainless-steel pins)
98 and lab (micropipette tips) materials described here draw us to the idea of a simple
99 "everywhere" electroanalysis that could be performed by non-qualified personal. The
100 portability of the system makes it useful for environmental analysis, e.g. water and
101 wastewater in situ analysis. Then, we decided to evaluate this new electrochemical lab-
102 on-a-tip device for assessing the quality of water in terms of the presence of anionic
103 surfactants. They mainly come from household aqueous discharge, industrial
104 laundering and other cleansing operations. Surfactants are considered emerging

105 contaminants. They have negative effects on water quality since they tend to
106 congregate providing properties such as foaming, emulsification and particle
107 suspension. Moreover, surfactants can pass through wastewater treatment plants and
108 reach environmental and even drinking water. These compounds produces harmful
109 effects in aquatic flora and fauna, and also in human health [21]. Therefore, the
110 concentration of surfactant in water is an important parameter to assess the water
111 contamination and quality. In the United States, ionic surfactants (mostly anionic) are
112 about two thirds of the total surfactants used [22]. The standard analytical method
113 highlights anionic surfactants, such as sodium dodecyl sulphate (SDS), as the most
114 common substances present in water and it is based in their methylene blue (MB)
115 activity. Anionic surfactants interact with MB, a cationic dye, forming an ionic pair. This
116 complex is transferred from the aqueous solution into an immiscible organic solvent
117 upon equilibration. The blue color intensity obtained in the organic phase is measured
118 spectrophotometrically and is proportional to the concentration of anionic surfactants.
119 This interaction has been applied also inversely, to adsorb MB (dye discharged to the
120 environment from textiles, dyeing and printing industries) on SDS-modified zeolites
121 [23]. We have applied a modification of this standard analytical method in which a
122 reduction in the sample volume and, henceforth, in the use of reagents is achieved
123 [24].

124 Moreover, instead of using a colorimetric assay, we have taken advantage of the
125 electrochemical activity of MB and its growing interest in biosensing (e.g. as indicator of
126 DNA hybridization processes through its interaction with the double strand [25]). Then,
127 we have adapted the modified standard method to detect electrochemically MB,
128 directly in the micropipette tip, as a way of determining SDS, a MBAS (methylene blue
129 active substances) frequently found in wastewaters. Then, when a constant
130 concentration of MB is added to the sample, its residual concentration in the aqueous
131 phase, after interaction with MBAS and further extraction to an organic media, is
132 indicative of the initial MBAS concentration in the sample.

133 Additionally, and considering the influence of surfactants on carbon electrodes,
134 which can improve electrochemical processes [26–28], a simplified electrochemical
135 method for the determination of SDS without following the extraction protocol
136 previously indicated, was also developed. In this way, an increment in the
137 electrochemical signal of MB (fixed concentration), seen for increasing SDS
138 concentrations, is the basis for its determination. Then, in this work, we report two
139 simple alternatives for SDS determination in water samples using an innovative and
140 low-cost multifunctional electrochemical platform.

141 **2. Material and methods**

142 2.1. *Chemicals and materials*

143   Through all this work, 100-1000 µL polypropylene micropipette tips were used
144 (Labbox Labware). For the assembly of the system and to facilitate micropipette
145 handling, a foot stand with a three-point gripper fastening tong was employed. As
146 electrodes, 26 x 0.59 mm stainless-steel pins (Metalurgica Folch) were selected.
147 Carbon paste (Gwent Group Ref. C10903P14) and N,N-dimethylformamide (DMF,
148 Merck) were employed for the fabrication of the working electrode. Acidic solutions of
149 different concentrations of methylene blue (Sigma Aldrich) were prepared in 0.5 M HCl
150 (hydrochloric acid 37% from Merck). Working SDS solutions for optimization studies
151 were prepared in ultrapure water. Also, chloroform (trichloromethane, Sigma-Aldrich)
152 was used for the analysis of anionic surfactants. Millipore Direct-Q(R) 3 UV was used
153 to obtain purified water.

154 2.2. *Electrochemical "in-the-tip" cell design*

155   We used stainless-steel pins as electrodes. The use of this kind of pins as
156 electrodes has been reported previously [6,7], including the stability of a stainless-steel
157 pin acting as reference electrode by comparison with a saturated calomel electrode (a
158 variation not higher than 3 mV over 30 min). The various parts of the pins were used
159 for different purposes, all of them schematized in Figure 1A. First of all, the stainless-
160 steel pins used as working electrodes (WE) were modified with carbon ink. Carbon
161 paste was diluted with N, N-dimethylformamide (50% w/w of carbon paste in DMF) to
162 generate the carbon ink for the modification. The mixture was vortexed for a few
163 seconds and then sonicated (37 kHz of frequency and 320 W of power) for 1 h.
164 Stainless-steel pins were mass modified adhering them, by their head, to a piece of
165 double-sided tape. Then, the stems were painted thrice with the carbon ink using a
166 brush (Figure 1B). Between the two first coatings, the pins were introduced in the oven
167 at 70°C for 15 min. After the last one, they were left for 1 h at 70°C.

168   Once the WEs were ready, two bare stainless-steel pins (one acting as reference
169 electrode, RE, and the other one as counter electrode, CE) were inserted in the
170 micropipette tip at a distance of 28 and 30 mm from the bottom, respectively. To favor
171 this, the micropipette tip was previously introduced in the oven at 70°C for 2 min. After
172 this, a volume of sample was aspirated, and the micropipette tip was sealed at the
173 bottom by introducing a pin covered with carbon ink (the WE). This process is
174 schematized in Figure 1C.

175   As the diameter of the pin stem is approximately half that of the end of the tip (0.59

176   and 1 mm, respectively) and to ease the handling of the device, the micropipette was

177   placed vertically, with the head of the pin acting as WE contacting a conductive copper

178   adhesive tape piece (3" Core, ½" (W) x 54 ft (L), Electron Microscopy Sciences)

179   manually cut (5 x 25 mm) and located over the bench. Crocodile clamps were

180   employed for connecting the copper tape (and then the WE) and the other two pins

181   acting as electrodes to the potentiostat (Figure 1C). The device was clamped to a

182   stand with a rod (as can be seen in Figure S1).

183   2.3.   *Electrochemical measurements*

184   All measurements were done using a µAUTOLAB TYPE III (Metrohm)

185   potentiostat. A volume of 360 µL of the working solution was aspirated into the tip to

186   record cyclic (CV) or linear sweep (LSV) voltammograms, between +0.5 and -0.6 V.

187   For both techniques, a scan rate of 0.1 V·s^{-1} and a step potential of 0.002 V were used.

188   2.4.   *Determination of SDS (MB active substance) after extraction of the ion pair*

189   *formed with MB*

190   A modification of the standard method used for the determination of anionic

191   surfactants as Methylene Blue Active Substances (MBAS) already described [24] was

192   here employed. In this work, 1 mL of the sample (i.e. SDS solution in ultrapure water

193   for optimizations or in tap water for real sample analysis) was mixed with 1 mL of a MB

194   stock solution (25 µg·mL^{-1} in 0.5 M HCl) and 1 mL of chloroform (CHCl$_3$). After shaking

195   for 30 s, the phase separation took place. Usually is the absorbance of the organic

196   phase containing the ion pair that is measured at 652 nm. But in this case,

197   electrochemical measurements were performed in the aqueous phase located over the

198   chloroform layer, by aspirating it into the tip and a LSV was recorded as commented in

199   the previous section.

200   2.5.   *Determination of SDS without extraction.*

201   In this case the procedure does not involve any extraction with chloroform. SDS

202   influences the electrochemical signal of MB, and this was used to determine its

203   concentration. Here, 360 µL of a solution containing SDS (in different concentrations)

204   and a fixed concentration of MB (25 µg·mL^{-1}) were aspirated to record the LSV as

205   commented in a previous section. The reduction current of MB changed according to

206   the concentration of SDS in solution. This allowed to obtain a calibration plot for SDS

207   concentration.

208   **3. Results and discussion**

209   3.1.   *Electrochemical design and characterization of the platform*

210   The design of the electrochemical cell is based on the use of very cheap and

211   available elements: pins and tips. Micropipette tips are also very versatile in regard to

212   the size (volume) and materials. In this case we chose commercial polypropylene tips

213   of 100-1000 µL volume, but a different polymer could be employed (even modified) and

214   pins could be inserted in tips of lower/higher volume to adjust the volume of the

215   electrochemical cell according to the requirements of the application. Pins are

216   introduced unmodified in the upper part of the tip (RE and CE) but, depending on the

217   volume, this height could be varied to ensure there is always ionic contact between the

218   three electrodes. Even being very simple, the different parts of the pin can be used for

219   different purposes. Hence: i) the sharp end allows to drill the polymeric tip material to

220   insert horizontally the RE and CE. In this way, there is no need to make holes in the tip

221   wall to introduce wires and, in turn, the addition of glue is not required. This simplifies

222   the procedure and saves time since glue curing is not needed. Moreover, as a positive

223   pressure has to be applied, the holes made are fully closed after insertion of the pins

224   and the pressure required to aspirate solutions with the micropipette is maintained. ii)

225   The stem is used directly as electrode (as for thread microfluidics [5]; in other previous

226   works the electrode was the pinhead [6–8]), either modified with carbon ink (WE) or

227   unmodified (RE and CE). In the last case, the part of the stem that is out of the tip can

228   be used to connect the pins to the potentiostat using crocodile clips. iii) The head of the

229   pin that is introduced vertically (WE) closes the tip at the bottom. With this design, the

230   connection is easily made by contacting the pinhead with copper tape that is adhered

231   on a flat substrate. The area of the WE could be changed by varying the length of the

232   pin. In our case, we used 26-mm long stainless-steel pins. With a diameter of 0.59 mm,

233   an approximated geometric area of 48.2 mm^2 was estimated (only the lateral area of

234   the cylinder).

235   After the three electrodes were ready inside the micropipette tip, the new

236   electrochemical platform was characterized. For this purpose, the electrochemical

237   behavior of methylene blue (MB) was studied. To be oxidized, MB has to be first

238   reduced to the leuco form [29]. In this redox process protons are involved, with

239   oxidation favoured at acid pH. Then, hydrochloric acid was chosen as electrolyte for

240   the study. A volume of 360 µL of a 25 µg·mL^{-1} MB solution in 0.5 M HCl was aspirated

241   and a CV was recorded. As can be seen in Figure 2A, a reversible redox process can

242   be observed with anodic and cathodic peak potentials at –0.02 and –0.08 V,

243   respectively.

244   The intensity of the cathodic peak current was chosen as analytical signal. The
245   precision between different pin-based WEs and different tip-based cells was studied
246   recording LSVs in a 25 µg·mL^{-1} MB solution in 0.5 M HCl. When LSVs were performed
247   using five different carbon ink pins-based WEs (washing with electrolyte between
248   measurements) and maintaining the same micropipette tip and the same RE and CE, a
249   relative standard deviation (RSD) of 5% for the cathodic peak current was obtained.
250   The RSD obtained when the whole tip-based cell was changed (n = 5) was 7% for the
251   cathodic peak current. These values demonstrated the adequate precision of the
252   developed electrochemical device.

253   3.2.   *Effect of SDS in the redox process of MB*

254   As a proof-of-concept, we proved the suitability of this new low-cost
255   electrochemical cell to perform simple and sensitive determinations of molecules of
256   interest. As it was reported in previous works, submicellar concentrations of surfactants
257   (cationic or anionic) produced an increase in the intensity of the oxidation and reduction
258   peak currents as well as in the reversibility of the electrochemical processes of
259   aromatic species [30–33]. In those cases, the surfactant is introduced as a modifier of
260   the carbon paste electrode (previously immersed in a surfactant solution [30,31,33]), or
261   by further addition of the surfactant to the measurement solution [32] (approach
262   followed in this work). Thus, the effect of SDS on the behavior of MB was studied
263   recording LSVs with the micropipette tip-based cell in presence and absence of SDS
264   (Figure 2B). The concentration of SDS (20 µg·mL^{-1}, i.e. $7.0 \cdot 10^{-5}$ M) was below the
265   critical micellar concentration (2.4 mg·mL^{-1} in water [34]). MB was in slight excess
266   compared to SDS (25 µg·mL^{-1}, $7.8 \cdot 10^{-5}$ M). As expected, a notorious increase in the
267   cathodic peak current (from -17.84 to -29.83 µA) was observed when SDS was present
268   in the measurement medium. Taking advantage of this, calibration curves performed by
269   varying the concentration of MB between 0.5 and 30 µg·mL^{-1} in both cases (Figure 2C),
270   showed a sensitivity two-fold higher when this concentration of SDS was present in the
271   medium. Slopes of -1.71 (R^2 = 0.996) and -0.84 µA (µg·mL^{-1})$^{-1}$ (R^2 = 0.996) were
272   obtained with and without SDS respectively. In Figure 2C, it can also be observed that
273   a wider linear range was achieved when SDS was added to the solution. This
274   improvement is likely due to the formation of the ion pair between the MB and the
275   surfactant, that is stabilized at the electrode surface (as in the case of SDS and
276   catecholamines [30] or cationic surfactants with flufenamic acid [33] or nitrophenol
277   [32]).

278   3.3.   *Determination of SDS using MB as indicator by both approaches: with and*
279   *without extraction*

280   High concentrations of surfactants could have important environmental

281   consequences and their determination becomes relevant in water samples suspected

282   of their presence. Based on previous results and taking advantage of the effect of SDS

283   in the cathodic peak of MB (approach I in Figure 3A), a calibration curve for SDS was

284   performed adding different SDS concentrations to a solution containing the same MB

285   concentration (25 µg·mL^{-1}). As Figure 3B shows, the intensity of the cathodic peak

286   current of MB (25 µg·mL^{-1}) increases with SDS concentration, following the equation

287   I_{MB} (µA) = −0.26 C_{SDS} (µg·mL^{-1}) − 9.56, with a R^2 of 0.994. The limit of detection (LOD)

288   and the limit of quantification (LOQ) were calculated as 3s_b/m and 10s_b/m respectively,

289   where m is the slope of the calibration plot, and s_b is the standard deviation of the

290   intercept. LOD and LOQ values thus obtained were 1.3 µg·mL^{-1} (4.5·10^{-6} M) and 4.3

291   µg·mL^{-1} (1.5·10^{-5} M) respectively.

292   A second approach for SDS determination (approach II in Figure 3A) was

293   developed modifying the standard method to detect anionic surfactants [23]. This is

294   based on the spectrophotometric measurement performed in the organic phase, which

295   contains the ion pair SDS-MB. In this work we have only considered SDS since is the

296   anionic surfactant usually present at higher concentration [22], but all the anionic

297   surfactants that form ion pairs with MB (MBAS) could be determined jointly. After

298   following the modified method explained in the experimental section, an aqueous and

299   an organic phase were obtained after extraction with chloroform and phase separation.

300   The organic phase contains the ionic pair MB-SDS and in the aqueous phase remains

301   the excess of MB that has not reacted. In this case, the aqueous phase was aspirated

302   inside the micropipette tip-cell for performing the electrochemical measurement. As the

303   ion pair has been extracted, the analytical signal (intensity of the peak current of the

304   cathodic process of MB) will be indirectly proportional to the concentration of anionic

305   surfactants (SDS) in the sample.

306   For this study, different concentrations of SDS were tested using a fixed

307   concentration of MB (25 µg·mL^{-1}). Each SDS solution (in ultrapure water) was mixed

308   for 30 s with 1 mL of MB solution and 1 mL of chloroform as explained in the Section

309   2.4. After phase separation, the layer at the top was aspirated into the micropipette-tip-

310   cell and the micropipette was clamped to the stand to proceed with the measurements.

311   As can be seen in Figure 3C, as SDS concentration increases, aqueous and

312   organic phases (top and bottom) become lighter and darker respectively, due to the

313   interaction of MB with SDS and further extraction with chloroform. In Figure 3D, a

314   calibration graph with the measurements performed in the aqueous phase after phase

315   separation for six different concentrations of SDS is shown, where I_{MB} is the intensity of

316 the MB cathodic peak current obtained from a LSV recorded from +0.5 V to -0.6 V. As

317 can be observed, the higher the SDS concentration, the lower the concentration of the

318 remaining MB in aqueous phase and, in turn, the analytical signal. Thus, this intensity

319 correlated linearly with the concentration of SDS following the equation I_{MB} (µA) = 0.37

320 C_{SDS} (µg·mL^{-1}) − 13.78 with a R^2 of 0.997. A LOD of 1.2 µg·mL^{-1} (4.2·10^{-6} M) and a

321 LOQ of 3.7 µg·mL^{-1} (1.3·10^{-5} M) were obtained using the above formula. Therefore,

322 similar LODs were obtained for both methodologies, although the measurement after

323 the extraction provides a slightly higher sensitivity (almost one and a half times).

324 3.4. *Application to tap water samples analysis*

325 In order to evaluate the accuracy of this new tip-based electrochemical device,

326 both methods (with and without previous extraction) were probed with spiked water

327 samples. Related to the possible interferences in real samples, two main cases could

328 be considered. On the one hand, any compound that could be reduced at the potential

329 of MB or lower could be considered as interferent. However, reduction processes in

330 organic molecules are not as common as the oxidation ones, and the potential of

331 reduction is not extremely negative. Moreover, instead of a chronoamperogram, a

332 linear sweep or cyclic voltammogram is recorded, that could be more informative about

333 the processes present. On the other hand, any anionic compound that could interact

334 with cationic methylene blue could decrease its content in solution and behave as

335 interferent when extraction is performed. This is the reason why the standard method

336 on which we are based for SDS determination is reported as method for MBAS

337 determination, in order to consider all the compounds. Similarly, when no extraction is

338 carried out, other surfactants could increase the signal of MB. However, SDS is one of

339 the main MBAS and the most common anionic surfactant. In case important

340 interferences are suspected, a thorough study should be performed previously.

341 Tap water from a municipality (Lugones, Siero) located in the center of the

342 Principality of Asturias, Spain, was spiked with two different SDS concentrations (8 and

343 17 µg·mL^{-1}). Samples were analyzed by both methodologies and the results obtained

344 are reported in Table 1. Using the equation of the calibration plot obtained with SDS

345 standard solutions prepared in ultrapure water, recoveries between 93% and 111% for

346 both concentration levels and both methods were obtained showing the feasibility of

347 the tip-based cell and not important matrix effects of the method. The mean values

348 obtained following both methodologies were statistically compared through a Student's

349 *t*-test: the *t* values calculated for the samples were lower than the tabulated t value for

350 two degrees of freedom and a 0.05 significance level. Hence, we concluded that there

were no significant differences between the values for SDS concentrations determined following the two different procedures, demonstrating the accuracy of the methodology.

Table 1. Results of the recovery test in tap water samples for both methods for SDS determination (mean value ± standard deviation for three replicates).

		[SDS] added ($\mu g \cdot mL^{-1}$)	[SDS] found ($\mu g \cdot mL^{-1}$)	Recovery
Sample 1	With extraction	8	7 ± 1	93 %
	Without extraction	8	8 ± 1	98 %
Sample 2	With extraction	17	19 ± 1	111 %
	Without extraction	17	17 ± 1	98 %

4. Conclusions

In-field analysis needs portable and simple devices and electroanalytical platforms have demonstrated to be very appropriate. In this context, a new electrochemical cell was fabricated in a very simple way using a micropipette tip and stainless-steel pins. Its good electroanalytical performance was demonstrated using methylene blue as known redox probe. Moreover, this novel tip-based cell was applied to real environmental analysis. It was applied to anionic surfactant determination, very relevant from the environmental point of view. SDS is a methylene blue active substance (MBAS) and can be determined indirectly measuring MB by voltammetry, which presents a reversible redox process at low potentials. Two approaches for SDS determination demonstrate the versatility of the tip-based cell to obtain accurate and precise results when both methods are compared.

The materials used are commonly found in most laboratories. Therefore, this do-it-yourself device becomes easily available worldwide. Tips and pins, that are mass produced, could be easily mass modified adding e.g., nanomaterials to the carbon ink of the working electrode, improving the final signal and obtaining better sensitivity. The availability of different types of micropipette tips and stainless-steel pins increases the possible applications of these versatile platforms.

Author Contributions

The manuscript was written through contributions of all authors.

Acknowledgment

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380 **References**

381 [1] A.A. Kumar, J.W. Hennek, B.S. Smith, S. Kumar, P. Beattie, S. Jain, J.P.
382   Rolland, T.P. Stossel, C. Chunda-Liyoka, G.M. Whitesides, From the bench to
383   the field in low-cost diagnostics: Two case studies, Angew. Chemie - Int. Ed. 54
384   (2015) 5836–5853. https://doi.org/10.1002/anie.201411741.

385 [2] D. Quesada-González, A. Merkoçi, Mobile phone-based biosensing: An
386   emerging "diagnostic and communication" technology, Biosens. Bioelectron. 92
387   (2017) 549–562. https://doi.org/10.1016/j.bios.2016.10.062.

388 [3] J. Yang, J. Zhou, Z. Lv, W. Wei, H. Song, A real-time monitoring system of
389   industry carbon monoxide based on wireless sensor networks, Sensors. 15
390   (2015) 29535–29546. https://doi.org/10.3390/s151129535.

391 [4] J. Adkins, K. Boehle, C. Henry, Electrochemical paper-based microfluidic
392   devices,   Electrophoresis.   36   (2015)   1811–1824.
393   https://doi.org/10.1002/elps.201500084.

394 [5] A.C. Glavan, A. Ainla, M.M. Hamedi, M.T. Fernández-Abedul, G.M. Whitesides,
395   Electroanalytical devices with pins and thread, Lab Chip. 16 (2016) 112–119.
396   https://doi.org/10.1039/C5LC00867K.

397 [6] E.C. Rama, A. Costa-García, M.T. Fernández-Abedul, Pin-based
398   electrochemical glucose sensor with multiplexing possibilities, Biosens.
399   Bioelectron. 88 (2017) 34–40. https://doi.org/10.1016/j.bios.2016.06.068.

400 [7] E.C. Rama, A. Costa-García, M.T. Fernández-Abedul, Pin-based flow injection
401   electroanalysis,   Anal.   Chem.   88   (2016)   9958–9963.
402   https://doi.org/10.1021/acs.analchem.6b01609.

403 [8] A. García-Miranda Ferrari, O. Amor-Gutiérrez, E. Costa-Rama, M.T. Fernández-
404   Abedul, Batch injection electroanalysis with stainless-steel pins as electrodes in
405   single and multiplexed configurations, Sensors Actuators, B Chem. 253 (2017)
406   1207–1213. https://doi.org/10.1016/j.snb.2017.07.148.

407 [9] P.I. Nanni, A. González-López, E. Nunez-Bajo, R.E. Madrid, M.T. Fernández-
408   Abedul, Staple-Based Paper Electrochemical Platform for Celiac Disease
409   Diagnosis,   ChemElectroChem.   5   (2018)   4036–4045.
410   https://doi.org/10.1002/celc.201800743.

411   [10]   T. Yuhi, N. Nagatani, T. Endo, K. Kerman, M. Takata, H. Konaka, M. Namiki, Y.
412   Takamura, E. Tamiya, Resin-based micropipette tip for immunochromatographic
413   assays in urine samples, J. Immunol. Methods. 312 (2006) 54–60.
414   https://doi.org/10.1016/j.jim.2006.02.011.

415   [11]   W. Zhang, Z. Chen, Preparation of micropipette tip-based molecularly imprinted
416   monolith for selective micro-solid phase extraction of berberine in plasma and
417   urine   samples,   Talanta.   103   (2013)   103–109.
418   https://doi.org/10.1016/j.talanta.2012.10.014.

419   [12]   T. Du, J. Cheng, M. Wu, X. Wang, H. Zhou, M. Cheng, Pipette tip-based
420   molecularly imprinted monolith for selective micro-solid-phase extraction of
421   methomyl in environmental water, Anal. Methods. 6 (2014) 6375–6380.
422   https://doi.org/10.1039/c4ay00766b.

423   [13]   A. González-López, M.C. Blanco-López, M.T. Fernández-Abedul, Micropipette
424   Tip-Based Immunoassay with Electrochemical Detection of Antitissue
425   Transglutaminase to Diagnose Celiac Disease Using Staples and a Paper-
426   Based   Platform,   ACS   Sensors.   4   (2019)   2679–2687.
427   https://doi.org/10.1021/acssensors.9b01096.

428   [14]   A.L.S. Fernandez, J.A.G. Calzón, A.C. Garcia, P.T. Blanco, Simple and reliable
429   fabrication of carbon fiber ultramicroelectrodes, Electroanalysis. 3 (1991) 413–
430   417. https://doi.org/10.1002/elan.1140030428.

431   [15]   R.A.B. da Silva, A.C. Rabelo, R.A.A. Munoz, E.M. Richter, Three-electrode-
432   integrated sensor into a micropipette tip, Electroanalysis. 22 (2010) 2167–2171.
433   https://doi.org/10.1002/elan.201000244.

434   [16]   D.W. Zhang, J.X. Liu, J. Nie, Y.L. Zhou, X.X. Zhang, Micropipet tip-based
435   miniaturized electrochemical device combined with ultramicroelectrode and its
436   application in immobilization-free enzyme biosensor, Anal. Chem. 85 (2013)
437   2032–2036. https://doi.org/10.1021/ac303223u.

438   [17]   X. Guo, Q. Wang, J. Li, J. Cui, S. Zhou, S. Hao, D. Wu, A mini-electrochemical
439   system integrated micropipet tip and pencil graphite electrode for detection of
440   anticancer drug sensitivity in vitro, Biosens. Bioelectron. 64 (2015) 594–596.
441   https://doi.org/10.1016/j.bios.2014.09.086.

442   [18]   S. Cinti, V. Mazzaracchio, G. Öztürk, D. Moscone, F. Arduini, A lab-on-a-tip
443   approach to make electroanalysis user-friendly and de-centralized: Detection of
444   copper   ions   in   river   water,   Anal.   Chim.   Acta.   1029   (2018)   1–7.

445   https://doi.org/10.1016/j.aca.2018.04.065.

446   [19]   R.L.   McCreery,   Advanced   carbon   electrode   materials   for   molecular
447   electrochemistry,   Chem.   Rev.   108   (2008)   2646–2687.
448   https://doi.org/10.1021/cr068076m.

449   [20]   A. Sanati, M. Jalali, K. Raeissi, F. Karimzadeh, M. Kharaziha, S.S. Mahshid, S.
450   Mahshid, A review on recent advancements in electrochemical biosensing using
451   carbonaceous   nanomaterials,   Microchim.   Acta.   186   (2019).
452   https://doi.org/10.1007/s00604-019-3854-2.

453   [21]   A.A. Siyal, M.R. Shamsuddin, A. Low, N.E. Rabat, A review on recent
454   developments in the adsorption of surfactants from wastewater, J. Environ.
455   Manage. 254 (2020) 109797. https://doi.org/10.1016/j.jenvman.2019.109797.

456   [22]   W.E.F. American Public Health Association, American Water Works Association,
457   Standard Methods for the Examination of Water and Wastewater, Public Health,
458   1999.

459   [23]   X. Jin, M. qin Jiang, X. quan Shan, Z. guo Pei, Z. Chen, Adsorption of methylene
460   blue and orange II onto unmodified and surfactant-modified zeolite, J. Colloid
461   Interface Sci. 328 (2008) 243–247. https://doi.org/10.1016/j.jcis.2008.08.066.

462   [24]   S. Chitikela, S.K. Dentel, H.E. Allen, Modified method for the analysis of anionic
463   surfactants as methylene blue active substances, Analyst. 120 (1995) 2001–
464   2004. https://doi.org/10.1039/AN9952002001.

465   [25]   A. Erdem, K. Kerman, B. Meric, M. Ozsoz, Methylene blue as a novel
466   electrochemical hybridization indicator, Electroanalysis. 13 (2001) 219–223.
467   https://doi.org/10.1002/1521-4109(200103)13:3<219::AID-ELAN219>3.0.CO;2-
468   7.

469   [26]   G. Tigari, J.G. Manjunatha, A surfactant enhanced novel pencil graphite and
470   carbon nanotube composite paste material as an effective electrochemical
471   sensor for determination of riboflavin, J. Sci. Adv. Mater. Devices. 5 (2020) 56–
472   64. https://doi.org/10.1016/j.jsamd.2019.11.001.

473   [27]   G. Tigari, J.G. Manjunatha, C. Raril, N. Hareesha, Determination of Riboflavin at
474   Carbon Nanotube Paste Electrodes Modified with an Anionic Surfactant,
475   ChemistrySelect. 4 (2019) 2168–2173. https://doi.org/10.1002/slct.201803191.

476   [28]   C. Raril, J.G. Manjunatha, A simple approach for the electrochemical
477   determination of vanillin at ionic surfactant modified graphene paste electrode,
478   Microchem. J. 154 (2020) 104575. https://doi.org/10.1016/j.microc.2019.104575.

479 [29] R. García-González, A. Costa-García, M.T. Fernández-Abedul, Methylene blue
480   covalently attached to single stranded DNA as electroactive label for potential
481   bioassays, Sensors Actuators, B Chem. 191 (2014) 784–790.
482   https://doi.org/10.1016/j.snb.2013.10.037.

483 [30] M.C. Blanco-López, M.J. Lobo-Castañón, A.J. Miranda Ordieres, P. Tuñón-
484   Blanco, Electrochemical behavior of catecholamines and related compounds at
485   in situ surfactant modified carbon paste electrodes, Electroanalysis. 19 (2007)
486   207–213. https://doi.org/10.1002/elan.200603712.

487 [31] S. Gutiérrez-Fernández, M.C. Blanco-López, M.J. Lobo-Castañón, A.J. Miranda-
488   Ordieres, P. Tuñón-Blanco, Adsorptive stripping voltammetry of rifamycins at
489   unmodified and surfactant-modified carbon paste electrodes, Electroanalysis. 16
490   (2004) 1660–1666. https://doi.org/10.1002/elan.200403032.

491 [32] T.B. Capelari, A.C. Pereira, L.L. Gonçalves de Oliveira, C.R. Teixeira Tarley,
492   Sensitive Simultaneous Determination of o-Nitrophenol and p-Nitrophenol in
493   Water by Surfactant-Mediated Differential Pulse Voltammetry, Anal. Lett. 52
494   (2019) 1462–1476. https://doi.org/10.1080/00032719.2018.1547311.

495 [33] I. Amor-García, M.C. Blanco-López, M.J. Lobo-Castañón, A.J. Miranda-
496   Ordieres, P. Tuñón-Blanco, Flufenamic acid determination in human serum by
497   adsorptive voltammetry with in situ surfactant modified carbon paste electrodes,
498   Electroanalysis. 17 (2005) 1555–1562. https://doi.org/10.1002/elan.200403262.

499 [34] H. Jakubowski, Biochemistry online: An approach based on chemical logic.
500   Lipids in water, 2020.

Figures captions

Figure 1. Schematic representation of the: A) different parts of the pin with their functions, B) modification of the stainless-steel pins used as working electrodes, C) electrochemical cell fabricated using a 100-1000 µL micropipette tip.

Figure 2. A) CV recorded in a 25 µg.mL^{-1} of MB in 0.5 M HCl (scan rate 0.1 V.s^{-1}, step potential 0.002 V) using the micropipette tip-cell. B) Cathodic processes recorded by LSV in a 25 µg.mL^{-1} MB solution without SDS and with 20 µg.mL^{-1} of SDS (scan rate 0.1 V.s^{-1}, step potential 0.002 V) using the micropipette tip-cell. B) Calibration curve of MB without SDS and with 20 µg.mL^{-1} of SDS. Error bars indicate the standard deviation of three replicates.

Figure 3. A) Scheme of both approaches (with and without extraction) for SDS determination using MB as indicator. B) Calibration graph employing the electrochemical measurements of MB obtained without extraction. C) Picture of microcentrifuge tubes with different SDS concentrations (0, 2.5, 5, 10, 20 and 30 µg. mL^{-1}) after phase separation using the modified standard method for the analysis of MBAS. D) Calibration graph employing the electrochemical measurements of MB obtained in the aqueous phase after performing the modified standard method for the analysis of MBAS. Each measurement was done in triplicate. Error bars correspond to the standard deviation of the measurements.
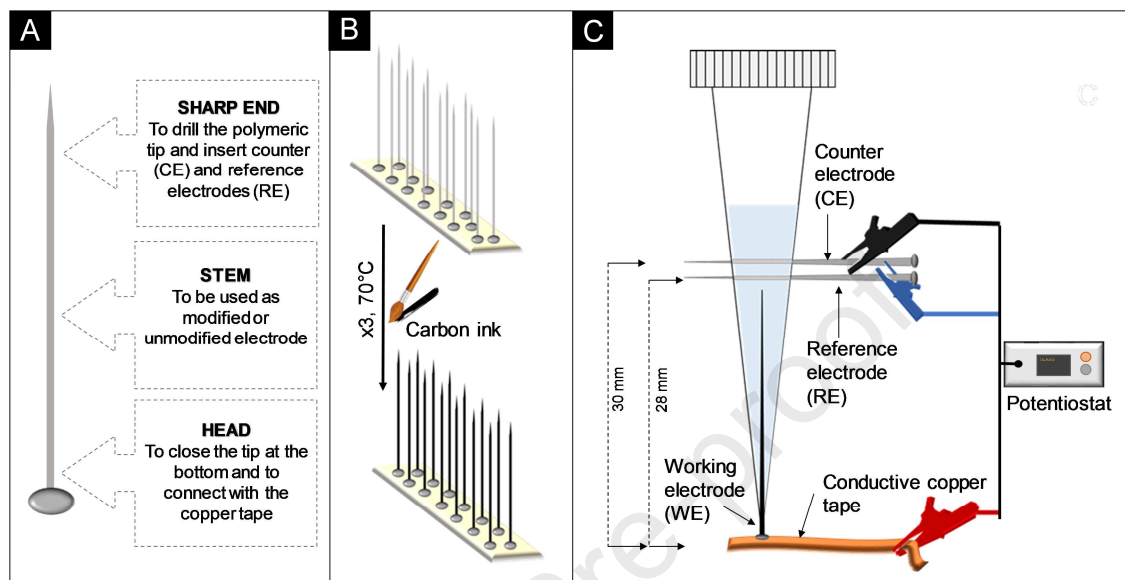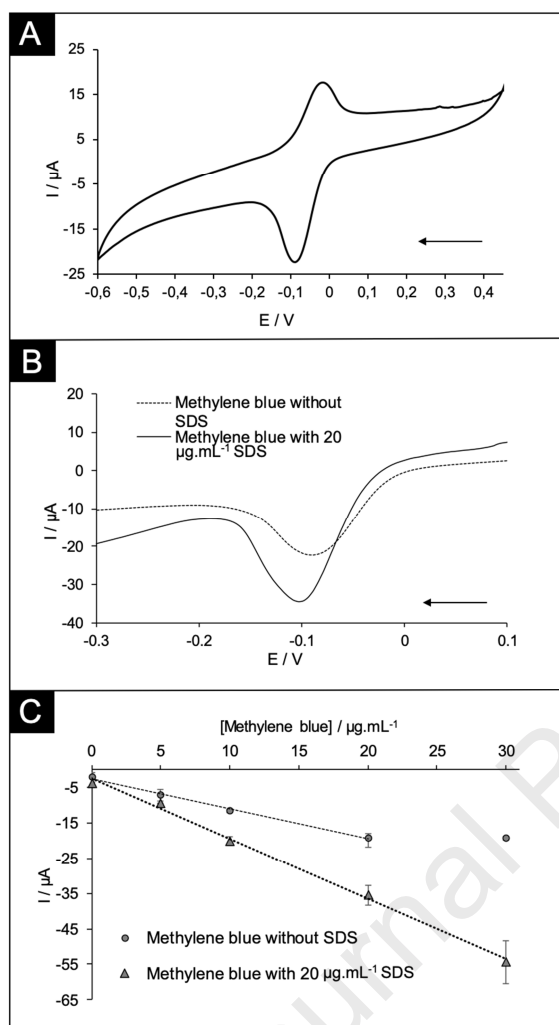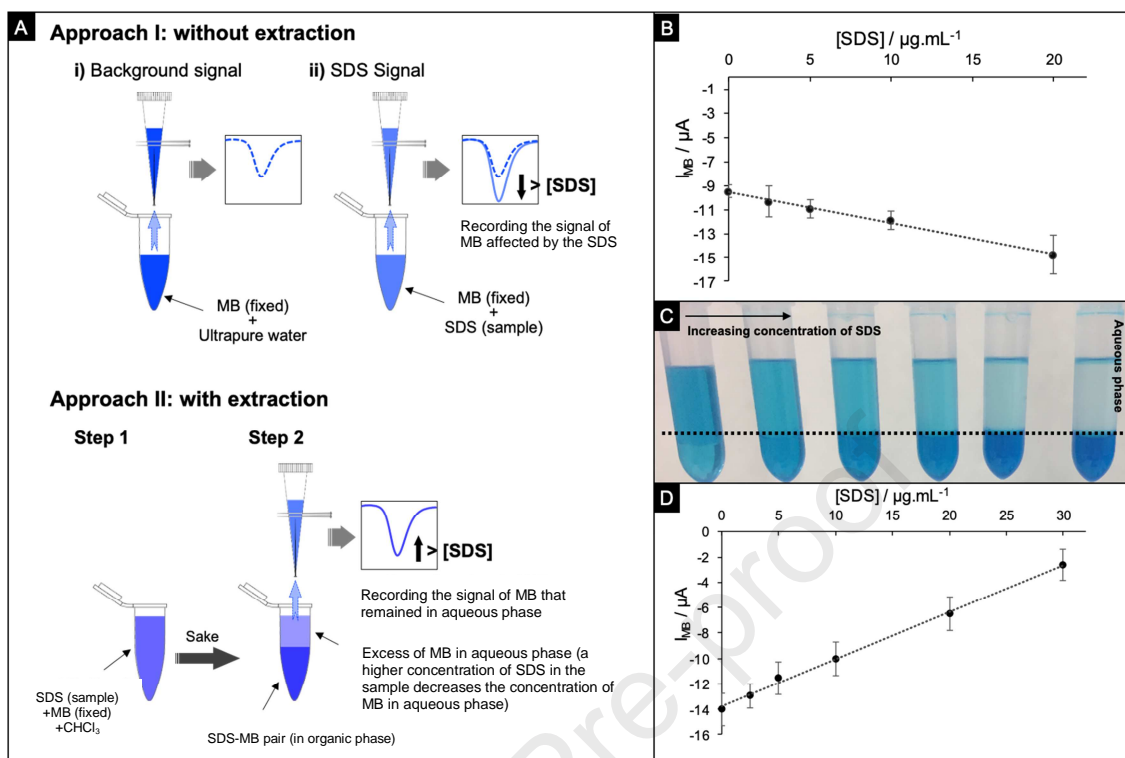
Figures

Figure 1

Figure 2

Figure 3

- Simple pin-based design for an electrochemical cell employing a micropipette-tip
- Low-cost electroanalysis using stainless-steel pins and micropipette tips
- Fast analysis of surfactants using micropipette-tip based electrochemical cells
- Electrochemical determination of SDS using a low-cost approach using pins and tips.
- Water analysis for electrochemical determination of surfactants

Declaration of interests

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