




Research Article

A cellulose-based colour test-strip for equipment-free drug detection on-site: application to sulfadiazine in aquatic environment



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Abstract

This work develops a simple and innovative test-strip to monitor antibiotics in aquaculture facilities by an equipment-free approach. It consists of a low-cost disposable cellulose paper that was chemically modified to produce a colour change when in contact with a given antibiotic. In brief, the cellulose substrate was subject to oxidation with periodate, followed by amination with chitosan binding and modification with Cu(II). The test strip was then dipped in the target solution and the intensity of the colour generated therein revealed the concentration of antibiotic present for concentrations higher than 0.5 mM. The higher the concentration in sulfadiazine (SDZ), the more intense the pink colour formed in the final solution, which was also turbid due to the insolubility of the formed product. This colour intensity also varied linearly with the logarithm of the SDZ concentration (from 0.5 to 5 mM), when plotted against the sum of the RGB coordinates extracted from digital pictures. The linear equation of this response was represented by $(R + G + B) = -256.1 \log(\text{SDZ, mol/L}) - 362.0$, with an R-squared of 0.9913. The test-strip was stable for at least 15 days and was selective in the presence of tetracycline and difloxacin, while the response to other members of the sulfadiazine family requires prior evaluation. Overall, the test-strips developed herein are inexpensive and provide valuable (semi-) quantitative data for monitoring SDZ in waters, a most valuable approach to control and reduce the level of antibiotics in fish tanks, which in turn may reduce the costs of fish production and the environmental concerns linked to this practice. Moreover, the test strip uses a cellulose substrate that has little environmental impact upon discard.

Keywords Test-strip · Aquaculture · Copper (II) · Sulfadiazine · Cellulose

1 Introduction

There has been a progressive increase in fish consumption, thereby declining wild fish populations and supporting the implementation of aquaculture activities [1, 2]. The intensification of fish production in captivity brings numerous economic benefits but also comes with some risks [3]. One of such risks relates to the local spread of invasive species that are not native from the farming area, which

become dominant in the local ecosystem for being more resistant than the native species. Yet, such dominance may bring new microorganisms and unbalance the local biodiversity [1]. Another risk comes with the introduction of various chemicals in the local ecosystem, from effluents containing the by-products of fish food and pharmaceuticals used for veterinary purposes. Several pharmaceuticals have been used in the context of aquaculture, including antibiotics [4].

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Antibiotics are natural or synthetic compounds that suppress the growth of specific microorganisms [5], used in aquaculture to fight and prevent infectious diseases associated to farmed fish, a frequent and devastating problem of the sector [6, 7]. This practice ends up by introducing antibiotics in the aquatic environment, through the direct application of antibiotics in water, through fish feed and excrements generated by the cultivated species [8, 9].

However, the presence of antibiotics in the aquatic environment has been linked to episodes of resistance to pathogenic bacteria [10–15]. This is also an outcome of using the same antibiotic compounds in humans and in fish and raises serious public health concerns [16]. The antibiotics used in aquaculture belong to several groups of human use [6, 17], among which sulphonamides appear as a relevant target [7, 10].

Thus, the controlled use of sulphonamides by fish farmers becomes a key issue to reduce the environmental/health impact of the common use of antibiotics in aquaculture, which would benefit all, fish farmers in particular and the world population in general. It should guarantee the desired therapeutic effect for a minimum dose of antibiotic, while generating financial advantages (by reducing the costs with pharmaceutical products) and environmental advantages (by reducing the emission of drugs into the aquatic medium). The practical implementation of such strict control requires, however, an expeditious and cheap analytical method, capable of providing analytical data in real time (or near), *on-site*.

Different works have been described for sulphonamide determination, most of which employing chromatographic systems with UV/vis [18–24] or mass spectrometry [25–29] detection schemes. These are typically unable to fulfil the basic requirements of this strict control. There are also several electroanalytical methods described in the literature for the determination of sulphonamides, from which devices targeting sulfadiazine (SDZ) may be found in [30–36]. In general, these methods are more appropriate for local analysis, having suitable analytical features, but still require specific instrumentation. The same is applied to ELISA [37] or optical-based [38–40] methods employed in sulphonamide detection.

As far as we know, very few methods report the development of test-strips for antibiotics, which are equipment-free methods. This includes plasticized PVC strips, in which the incubation of a given antibiotic lead to a given colour, yielding semi-quantitative data by comparing with a standard colour palette [41, 42]. These works involved a colourimetric reaction between a target antibiotic and a suitable reagent, entrapped within a plasticized PVC support. Another test-strip for antibiotic involves a cellulose paper substrate that was further modified in a self-assembled monolayer process [43]. The use of a cellulose support

offers the advantage of leading to a detection system that is inexpensive and environmentally safe, while delivering waste materials that are not critical for the environment. Moreover, this test-strip operated in a similar way to the universal pH paper, providing intensified colour gradients for increasing antibiotic concentrations. Yet, none of these works is dedicated to sulphonamides.

Thus, the present work describes the development of a test-strip on a cellulose support aiming at the determination of sulphonamides by visual comparison of the colour changes. This concept is applied herein to SDZ, as the representative compound of the sulphonamide group. To this end, a suitable reaction is first identified in aqueous medium, aiming to identify a coloured reaction that is SDZ concentration dependent. This reaction is further adapted to a solid substrate, involving a suitable chemistry. The optimization and characterization of the test-strips are described, assessed by visual detection of the colour change or by handling the colour coordinates of the digital images. A practical application is also described.

2 Experimental section

2.1 Reagents and apparatus

All reagents were of analytical grade and deionized or ultra-pure water was used throughout. Iron chloride hexahydrate (Scharlau), lead nitrate (II) anhydrous (Riedel–deHaen), copper sulfate (II) pentahydrate (Panreac), zinc chloride (Merck), hexahydrate magnesium chloride (Analar normapur), aluminum sulfate 16-hydrate (BDH), ammonia (Sigma), ammonium chloride (Analar normapur), ammonium acetate (Analar Normapur), acrylamide (Sigma), *N*-tert-butylacrylamide (Sigma), *tris*(hydroxymethyl)aminomethane (TRIS, Fisher Bioreagents), triethanolamine hydrochloride (ED, Fulka), ethylenediaminetetraacetic acid (EDTA, Sigma), iodine (Riedel–deHaen), sodium metaperiodate (VWR) glutaraldehyde (Fluka), tetracycline (TEC, Applichem), SDZ (Sigma) sulfathiazole (STZ, Sigma), sulfamethoxazole (SMZ, Sigma), difloxacin (DIF, Solvay) were employed throughout. Cellulose paper was obtained from Fanoia (ReferenceS-300).

A Thermo Scientific Evolution 220 spectrophotometer was used for UV/Vis readings. A Fourier Transform Infrared (FTIR) spectrometer from Thermo Scientific, Nicolet IS10, coupled to an accessory of attenuated diffuse reflectance (ATR) was used to follow up the chemical modifications in the solid materials. Digital images from the test-strip/solutions were collected with a Canon Power shot, SX500IS, and the corresponding coordinates (RGB or HSL colour systems) extracted from the Paint program of Windows.

2.2 Reactions in aqueous medium

The possibility of forming a coloured product with SDZ was checked among several reactants, including permanganate, iron (III), lead (II), copper (II), aluminium (III), magnesium (II), zinc (II) and iodine. This preliminary reaction was tested by mixing 1.0×10^{-4} M SDZ with 1.0×10^{-4} M reactant solutions (1:1), all prepared in water. The resulting UV/Vis spectra were recorded between 360 and 780 nm.

The interaction of the previous reaction with amine-derived compounds was checked for different compounds, including EDTA, ammonia, ammonium acetate, acrylamide, *N-tert*-butylacrylamide, TRIS and triethanolamine. This study involved the preparation of 1.0×10^{-3} M copper sulphate, 1.0×10^{-2} M amine-based compound and 1.0×10^{-3} M SDZ standard solution. These solutions were mixed in equal parts (1:1:1) to check the effect of the amine compound on the coloured detection of SDZ. In control tests, the SDZ or the amine solution were replaced by water. The absorption spectra of the resulting mixtures were recorded from 360 to 780 nm.

2.3 Chemical modification of the cellulose substrate

The cellulose substrates were first cut in 1.0×1.0 cm², washed with ethanol and subject (sequentially) to oxidation, amination and modification with copper. All steps were followed by FTIR analysis.

The paper oxidation was achieved by reaction a 0.2 M sodium metaperiodate solution, having its pH adjusted to 4.37, by adding few drops of 1 M sodium hydroxide solution prior to completing the final volume with water. Each paper was then incubated individually in the periodate solution, on a dark bottle, under constant stirring side, for 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, and/or 24 h, using room temperature conditions or 40, 60 or 80 °C.

Amination used 1.0% chitosan solutions prepared in acetic acid 2% (w/w). Dissolution was completed by orbital shaking at 200 rpm, at a temperature of 70 °C [44]. Some tests included glutaraldehyde in the chitosan solutions, prepared to generate 10% glutaraldehyde in the final solution and completing to final volume with phosphate buffer 0.1 M, pH 7 [44]. These tests were made by (i) layer-by-layer deposition, in which chitosan, glutaraldehyde and copper were casted on the paper substrate sequentially and in this order; or (ii) crosslinking, in which glutaraldehyde and chitosan were mixed before casting, having the copper solution casted thereafter.

In (i), the dry oxidized paper was (1) covered by the glutaraldehyde solution and placed in the oven for 50 °C, until completely dry; (2) then covered by the chitosan solution, for 30 min; (3) the strips were then incubated in a 1.0×10^{-1} M copper (II) sulphate solution, in a closed dark container and

kept under stirring for 24 h, at room temperature. At this time, the papers were removed from the solution, washed with water and dried in an oven at a maximum temperature of 30 °C [44]. Finally, each test-strip was placed in a test tube and added of SDZ solution. This procedure was also repeated by skipping step (1). In (ii), the amination procedure was similar to that described before, but step (1) used a mixed solution of glutaraldehyde and chitosan, and step (2) was eliminated.

2.4 Optimizing the conditions for colour formation

The order of the reagents added as well as the effect of the final pH in the colour formation mixture were evaluated. For this purpose, (A) a copper sulphate solution of 1.0×10^{-2} M was added of a solution of ammonium acetate 2.0×10^{-2} M, let stand for 30 min and then added of SDZ solution of 1.0×10^{-3} M, all in equal parts (1:1:1); (B) the order of adding copper sulphate and ammonium acetate was inverted; (C) ammonium acetate was replaced by water; (D) SDZ was added first to ammonium acetate, and the copper solution was added after this. In the resulting solutions, the pH value was noted and small alterations were made to this solution, by adding few drops of hydrochloric acid or saturated sodium hydroxide solutions, always recording the final pH value.

2.5 Procedures for evaluating the test-strips

The performance of the test strips was evaluated by incubating each test-strip in a test tube containing 2 mL of the target SDZ solution, of varying concentrations, ranging 1.0×10^{-6} to 5.0×10^{-3} M. The cross-response to other antibiotics was tested by incubating the test-strips in a solution of 1.0×10^{-3} M of STZ, SMX, TEC or DiFL. A stability test was made by checking the response of the test-strips stored at room temperature or in the refrigerator (4 °C) dipped in water, after 15 days. The test-strips were also used to analyse environmental waters from a non-controlled local well, spiked with SDZ to reach 3.0×10^{-3} , 9.0×10^{-4} and 7.0×10^{-4} M. All coloured results were recorded with a camera, after 30 min incubation, and the digital images compared with a homemade palette or used to extract the RGB/HSL coordinates [45], further handled mathematically to generate a linear correlation with log concentration and allow the production of quantitative information.

3 Results and discussion

To develop a test-strip that generates a colour when in contact with SDZ, it was necessary to identify a reaction that would lead to the production of a coloured change.

This could be either by the formation of a coloured product or by the decreasing concentration of a coloured reactant. The corresponding studies were made first in aqueous medium, and only later translated into a solid support, selecting at this time the best reaction.

3.1 Selecting the coloured-forming reactant

In the absence of reactions described in the literature for this purpose, and considering that many redox or complexation reactions lead to coloured products, different cationic species were tested. This included manganese (VII, as permanganate), iron (III), lead (II), copper (II), aluminium (III), magnesium (II), zinc (II) and molecular iodine (or I_3^-). The results obtained are shown in table S1 and evidence that only copper (II) yielded a colour change, comparing the initial colour of the reactant solution with the colour obtained after adding SDZ.

The absorbance spectra involved in the SDZ/Copper reaction are shown in Figure S1, evidencing that the absorption of the copper solution in the range from 650 to 800 nm decreased after adding SDZ, probably signalling a decrease in the Cu(II) concentration. Moreover, this was coupled to an absorption increase, starting at about 470 nm and down to lower wavelength values. Overall, this disclosed the formation of a coloured species in a region where the colour seen by human eye was reddish. Thus, the reaction between SDZ and Cu(II) was further optimized.

3.2 Monitoring the impact of amines

In general, Cu(II) establishes stable complexes with amine derived compounds, and this could be a simple way to bind it to a cellulose substrate [43]. However, this could be also a disturbing element if Cu(II) would bind to the amine groups with high affinity, thereby decreasing the amount of available copper for a subsequent reaction with SDZ. Thus, the same reaction was further tested in the presence of different amine-based reagents, aiming to identify a condition that would not affect the reaction taking place between SDZ and Cu(II). To this end, EDTA, ammonia, ammonium acetate, acrylamide, *N-tert*-butyl acrylamide, TRIS, and triethanolamine hydrochloride were tested. Different effects were observed herein, with EDTA and ammonium acetate generating the most opposite results. The interaction between EDTA and copper formed very stable complexes, subsequently limiting the reaction between Cu(II) and SDZ. This was evidenced by the absence of absorbance in 390–470 nm range (Figure S2) and by a significant increase in the wavelength range above 600 nm, just as expected. In contrast, the presence of ammonium acetate allowed occurring the reaction between EDTA and

copper (Figure S3), even under a more intense blued colour of the copper solution in the presence of ammonium acetate. This could also reflect the impact of the pH change upon the copper species in solution.

Overall, the results suggested that further tests could be done in the presence of ammonium acetate, if necessary, thereby avoiding a significant impact upon the target reaction while favouring the conditions for copper binding to the cellulose substrate.

3.3 Oxidation of the cellulose substrate

Before binding an amine-derived compound that would adsorb copper, the cellulose paper was pre-treated, to prepare it for this subsequent modification stage and to ensure that it would become a reproducible material throughout the different studies. A schematic representation about these modifications is shown in Fig. 1.

As pre-treatment, an oxidation stage was considered necessary as it would clean the substrate from side-compounds and yield carbonyl groups at the surface. These groups would be capable of establishing a covalent binding to any amine group. Periodate was selected for the purpose of paper oxidation [46]. Typically, periodate oxidizes carbon–carbon bonds when each of these carbons is attached to hydroxyl ($-OH$) and/or carbonyl ($=O$) groups. In cellulose, this takes place at C_2-C_3 of the pyranose unit, yielding the oxidation of each hydroxyl group up to carbonyl groups [46].

The extent of periodate oxidation depends, however, from the conditions selected for this reaction, including periodate concentration, time and temperature. Herein,

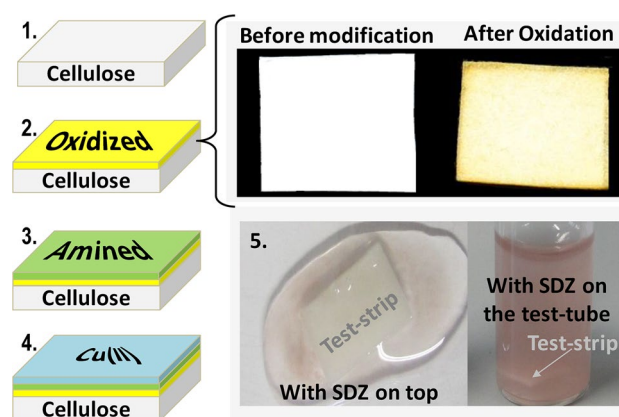


Fig. 1 Schematic representation of the several stages of cellulose paper modification. 1. Cellulose paper before modification. 2. Oxidation with periodate (pictures on the right exhibiting the images before and after oxidation). 3. Amination with chitosan. 4. Modification with Cu(II). 5. Addition of a SDZ solution (solution casted on top of the test-strip or deposited inside the test tube, evidencing the dispersion of a solid coloured precipitate)

the oxidation was carried out at room temperature (by 0.5, 1, 2, 4, 5, 6 and 24 h), at 40 °C (for 1, 2, 3 and 5 h), at 60 °C (for 1, 2 and 3 h) and 80 °C (for 1, 2 and 3 h), keeping the same periodate concentration. All these tests were followed by FTIR studies, and the relevant results are shown in Fig. 2. The spectrum of plain cellulose showed a broadband at 3300 cm^{-1} assigned to O–H stretching, and a strong peak at around 1100 cm^{-1} assigned to C–O stretching, both bands being typical of the cellulose structure. The chemical oxidation of glucose yielded the formation of carbonyl groups, typically located at $\sim 1640 \text{ cm}^{-1}$, which could be coupled to an alteration of the hydroxyl group absorption, in the region of 3300 cm^{-1} . Considering the tests carried out at room temperature, the formation of the carbonyl peak was only evident after 24 h reaction (medium peak intensity at 1637.8 cm^{-1}), with the paper remaining substantially unchanged until 6 h. In studies conducted at 40 °C, there was no evidence of a carbonyl group up to 5 h reaction, the maximum period evaluated for this temperature. For studies carried out at 60 °C, the carbonyl peak was already evident by the first hour of reaction (at $\sim 1638.6 \text{ cm}^{-1}$), and remained similar after 2 h (Figure S4). The extension of this reaction for 3 h damaged the paper, leading to the loss of several parts of the original structure. Studies at 80 °C supported further this possibility, since the papers were partially destroyed and there was no evident role of oxidation on any of the stages for this reaction time (Figure S5).

Thus, subsequent assays were made for a cellulose oxidation with periodic acid at 60 °C, for 2 h. The aspect of the so obtained paper is shown in Figure S6, where a yellowish colour (as aged paper) is visible, as well as a slight reduction of the paper size.

3.4 Amination of the oxidized substrate

The modification of the oxidized paper with an amine layer was considered next (Fig. 1) aiming to a steady copper (II) binding to the test-strip. To this end, chitosan was considered, as it is an amino polysaccharide and thereby would displaying affinity for another polysaccharide support. In order to improve its binding to the substrate, the addition of glutaraldehyde was also considered, as a possible reticulating agent. Thus, this study was made by adding only chitosan to the paper or adding both together or separately, being glutaraldehyde added first to act as an effective cross-linking system between chitosan and the oxidized paper.

In general, the presence of chitosan alone yielded a pale yellow colour, while the addition of chitosan and glutaraldehyde yielding a light brown colour (Figure S7). The subsequent deposition of copper on the aminated layer gave rise to a darker brown colour when glutaraldehyde was present or to a blue colour when only chitosan was present. From this study, it was presumed that a redox reaction between copper (II) and the aldehyde groups of glutaraldehyde could be taking place, which would further

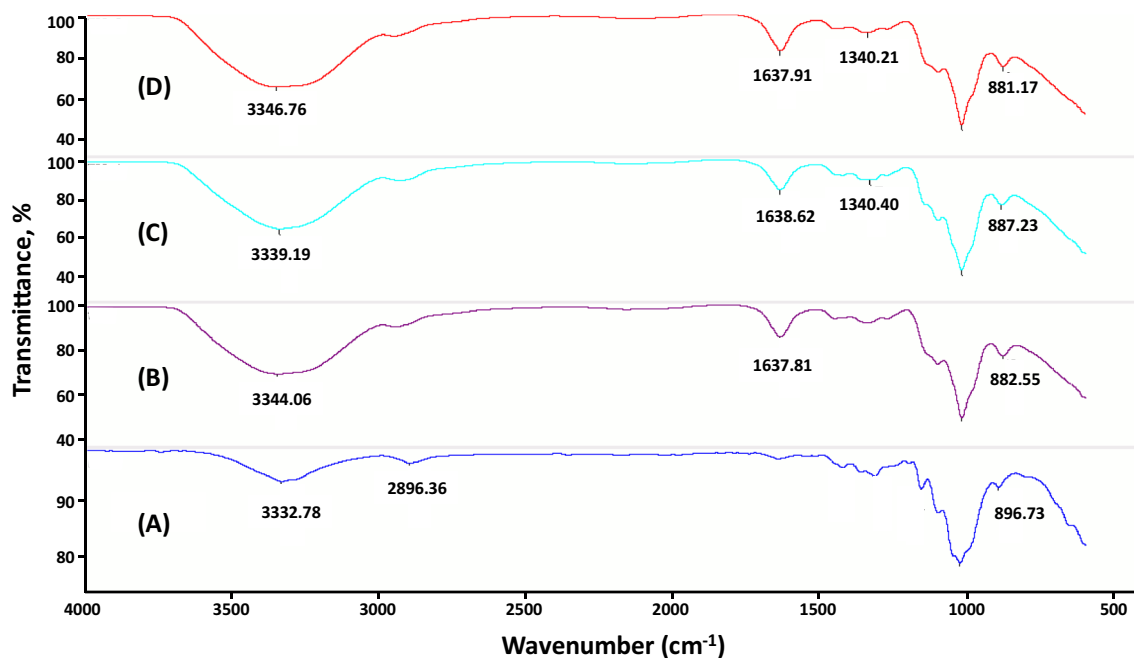


Fig. 2 FTIR spectra of the cellulose paper, as used (A) or modified by periodate oxidation, at room temperature for 24 h (B) or 60 °C for 1 (C) or 2 h (D)

hinder the detection of SDZ. This was confirmed by testing these papers with SDZ, from which it was clear that only the paper with chitosan alone yielded a colour change (Figure S8) and this colour change was also linked to the formation of a precipitate around the sensing material (Fig. 1). Overall, the amination of the oxidized paper was made with a chitosan film, from this point on.

3.5 Testing the order of the reagents and the pH

The pH value was a critical aspect for the success of the test-strips. In one hand, it determined the solubility of copper, which could limit the subsequent reaction with SDZ or its leaching into the solution. On the other hand, it also changes the protonation of the amine groups on the surface, which implies changes in the capacity of the surface to retain copper alone. Moreover, the reaction between SDZ and copper shall be pH dependent, as most colorimetric reactions are. Thus, it was important to evaluate the impact of pH and the impact of ammonium acetate selected previously, which could be likely omitted after the presence of chitosan. This was done as described in table S2, tested for concentrations of SDZ of 1.0×10^{-2} M.

In general, the mixture of copper with SDZ gave a pink coloured precipitate, as shown in Figure S9, at all pH values studied (~ 1 , 7 and 12). This precipitate was formed only when the ammonium acetate was absent or when it was added after copper. The addition of ammonium acetate before copper prevented the formation of a colour change. Thus, it became clear that ammonium acetate would bind copper and subsequently reduce the amount of copper available to react with SDZ.

3.6 Main features of the coloured product

The precipitate so formed was further isolated (Figure S10) and analysed, aiming to understand if it would result from redox or complexation reactions. First, the solid was added of a few drops of sulphuric acid, which originated its dissolution and the appearance of a bluish colour, visible to the naked eye and characteristic of copper (II) under these conditions (Figure S10). Thus, it was assumed that a redox reaction was not present. Moreover, and according to previous data [47], SDZ is expected to form a complex with the copper(II) ligand, in the form of $M(\text{SDZ})_2$, in which M^{2+} is the copper ion, which is not soluble in water. This is indeed consistent with the formation of a pink turbid solution, which has intensified colour for increasing SDZ concentrations. Moreover, this was also consistent with the FTIR data obtained for this solid (Figure S11), revealing a similar profile between SDZ and the precipitated material.

3.7 Test-strip response to SDZ concentration

The test-strip was further dipped in a wide range of SDZ concentrations, in order to identify the concentration range in which the test-strip would respond in a concentration dependent-manner, thereby giving rise to (semi-) quantitative data. The different colours were compared by taking a picture to all flasks, together, in order to normalize colour variations and ambient brightness between standard solutions and samples. Each digital image was read after in the "Paint" program of Windows®, in order to extract the coordinates from the colour system used therein, RGB and HSL.

The first tests made covered a wide range of concentrations, aiming to identify the region for which the papers would produce a colour change that was visually perceptible. The concentrations evaluated first for this purpose ranged from 5.0×10^{-3} to 1.0×10^{-6} M. To this end, the sensing papers were dipped in each standard solution and let stand there for 30 min. The results obtained after this are shown in Figure S12. In general, only concentrations higher than 5.0×10^{-4} M produced an apparent colour change. This change would translated into a turbid solution with a pink colour. This was an outcome of having the red precipitate being formed between SDZ and Cu(II), which leached from the sensing paper for being water insoluble and was overall dispersed through the solution medium. In another perspective, Cu(II) could be also leaching from the sensing paper into the SDZ solution, yielding the red precipitate in the aqueous medium. Overall, the presence of SDZ was revealed by the formation of a pink turbid solution.

After this, the SDZ concentrations were refined to establish a calibration profile of the sensing paper. The following values were selected for this purpose: 5.0×10^{-3} ; 4.0×10^{-3} ; 3.0×10^{-3} ; 2.0×10^{-3} ; 1.0×10^{-3} ; 9.0×10^{-4} ; 8.0×10^{-4} ; 7.0×10^{-4} ; 6.0×10^{-4} ; and 5.0×10^{-4} M (corresponding to C_1 to C_{10} tests indicated in Fig. 3). The results so obtained revealed the occurrence of visible changes that were also concentration dependent, as the turbidity of the solution increased for increasing concentrations.

A representative part of the digital image of each concentration was collected and subsequently imported to the program Paint, of Windows® (Fig. 3). From these, the average coordinates were collected from RGB and HSL systems and the average coordinates established. This would act as a future coloured palette with which any operator could compare to identify a semi-quantitative information.

3.8 Test-strip selectivity

The selectivity of the sensing material response to SDZ is a very important characteristic, considering the

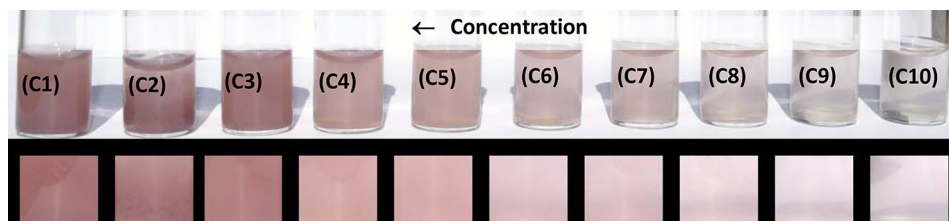


Fig. 3 Pictures of the test tubes with the test-strip and SDZ solutions of 5.0×10^{-3} (C1); 4.0×10^{-3} (C2); 3.0×10^{-3} (C3); 2.0×10^{-3} (C4); 1.0×10^{-3} (C5); 9.0×10^{-4} (C6); 8.0×10^{-4} (C7); 7.0×10^{-4} (C8);

6.0×10^{-4} (C9); e 5.0×10^{-4} (C10) M; and presentation of the real image to the various concentrations of SDZ, between 5.0×10^{-3} and 5.0×10^{-4} M

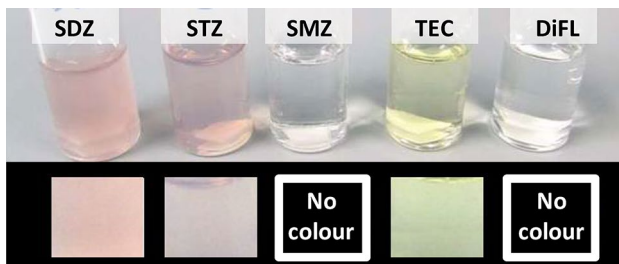


Fig. 4 Pictures of the test tubes with the sensing-SDZ paper and solutions of SDZ or possible interfering species (STZ, SMZ, TEC and DiFL), in concentrations of 1.0×10^{-3} M

subsequent analytical application. For this purpose, the sensing paper was placed in contact with other antibiotics employed in aquaculture. The antibiotics selected for this study were STZ, SMZ, TEC and DiFL. STZ and SMZ are sulphonamides (as SDZ), TEC is a tetracycline and DiFL is a quinolone. The corresponding chemical structures may be found in Figure S13.

As may be seen in Figs. 4, the typical pink turbid solution generated by the sensing paper on the presence of SDZ was completely different from the other compounds, except in the case of STZ. Overall, STZ is another sulphonamide, for which its reaction with Cu(II) could also be expected, although herein at a lower extent than SDZ, due to the lighter colour observed and the lower turbidity of the solution. Moreover, other sulphonamides may react with Cu(II) with different extents [48], which was the case of SMZ that yielded a transparent solution. Finally, TEC did not promote a colour change that could be misleading in the case of SDZ readings, and DiFL had no reaction with Cu(II).

In general, the sensor responded with good selectivity for SDZ and its use for testing other sulphonamides in the context of aquaculture could be further characterized. Antibiotics from other therapeutic groups were unable to lead to an interfering response.

3.9 Test-strip stability

The sensor stability was evaluated throughout time, for 15 days, stored at room temperature or in the refrigerator dipped in deionized water. The pictures of the results obtained are shown in Figure S14. As expected, it was verified that the sensing material stored in a refrigerator in deionized water had no response to SDZ (likely the copper ions were leached from the sensing paper). In contrast, the sensing paper stored at room temperature, for the same period, reacted with SDZ to produce the expected pink turbid solution, but with to a slightly lower extent. In a real case scenario, this may be solved by testing few standard solutions as controls in the same day the water samples are being analysed.

3.10 Application to water samples

The sensor was tested by monitoring SDZ in environmental waters from a well. These waters were spiked with SDZ, in 3.0×10^{-3} , 8.0×10^{-4} , and 7.0×10^{-4} M (identified by the acronyms Aca, Acb and Acc, respectively). The results are shown in Figure S15 and each of these reaction flasks were interface and aligned with the other test tubes of the calibration flasks, in order to anticipate their concentration range. As may be seen in Figure S15, bottom, there is a gradient colour change with the concentration, and the colours forming from the calibrating standard solutions may be further used to compare the observed colour in the sample and provide semi-quantitative information.

As before, the digital image of these flasks were read in the Paint program and their coordinates collected. This data was mathematically treated to generate a calibration curve profile, but it was very complex to find a linear trend. Thus, the coordinates were again collected at various sections of the reaction tube, both in the RGB and HSL systems. After several tests, which varied the reading points and the mathematical models applied to the coordinates, it was concluded that the most suitable setting would involve reading the colour at the interface between the liquid and the air, as shown in Fig. 5. In a general way, it

was found that the logarithm of the SDZ concentration varied linearly with the sum of the coordinates R, G and B. throughout the whole range of concentrations studied.

In general, this linear trend was used to provide more accurate information about the samples. The quantitative analysis of the tested environmental water samples yielded relative errors below 5.4% (Fig. 5), thereby suggesting the accuracy of the results obtained. The visual comparison of samples and standards also allowed measuring the same samples in a semi-quantitative way. Overall, the sensing paper is a useful tool to monitor in situ the water from the fish tanks in aquaculture facilities, aiming to quantify and follow-up closely the values of administered antibiotic.

4 Conclusions

This work describes the assembly of a test-strip sensitive to SDZ, obtained by simple chemical modification of a cellulose substrate. Copper (II) was found a suitable reagent to generate a coloured water insoluble product in the presence of SDZ. It was attached to a chitosan layer casted on cellulose.

The analytical characterization of the test-strip evidenced a coloured response that was SDZ concentration dependent, from 5.0×10^{-4} and 5.0×10^{-3} M. It provided a semi-quantitative information of water samples by comparison with an established colour palette, made with standard solutions. More accurate quantitative data could also be extracted by plotting the $\Sigma(R,G,B)$ of the images collected against the log concentration of SDZ.

Moreover, the sensor was found stable for a practical application under aquaculture facilities. Spiked samples analysed this way also provided relative errors $< 5.4\%$, even for samples of lower concentration.

In general, the test-strip was selective to SDZ when in the presence of antibiotics from other chemical families. The presence of another member of the sulfadiazine family had a coloured response but with lower intensity, while another member showed no response at all for the same concentration level. In principle, this indicated that the reaction undergoing in this test-strip involves the main chemical structure of the sulfadiazine family, but the substituents in the overall structure dictate the sensitivity by which each member of this family responds. Thus, in principle, this test strip may be extended to other sulphonamides, but this requires additional optimization to provide a reliable response to the other members of this family. In general, the test-strip developed herein offered advantageous features for a local monitoring of antibiotics in aquaculture water. The overall procedure is inexpensive, expeditious and quick, having no environmental impact. A comparison of this method to previous recently published chemical sensing systems [32, 35, 49–52] may be established by checking Table 1, highlighting that the herein proposed method is highly simple in terms of synthesis (summarized in Figure S16) and analytical application.

Moreover, it is important to highlight that the residues arising from this approach should be suitably treated before disposal. To this end, there are various solutions indicated in the literature, from which the use of photocatalytic materials is advised [53–55].

Fig. 5 Digital images of standard solutions (A), the corresponding calibration curve (B) and the theoretical and experimental concentration values of the samples, along with the corresponding relative errors (C)

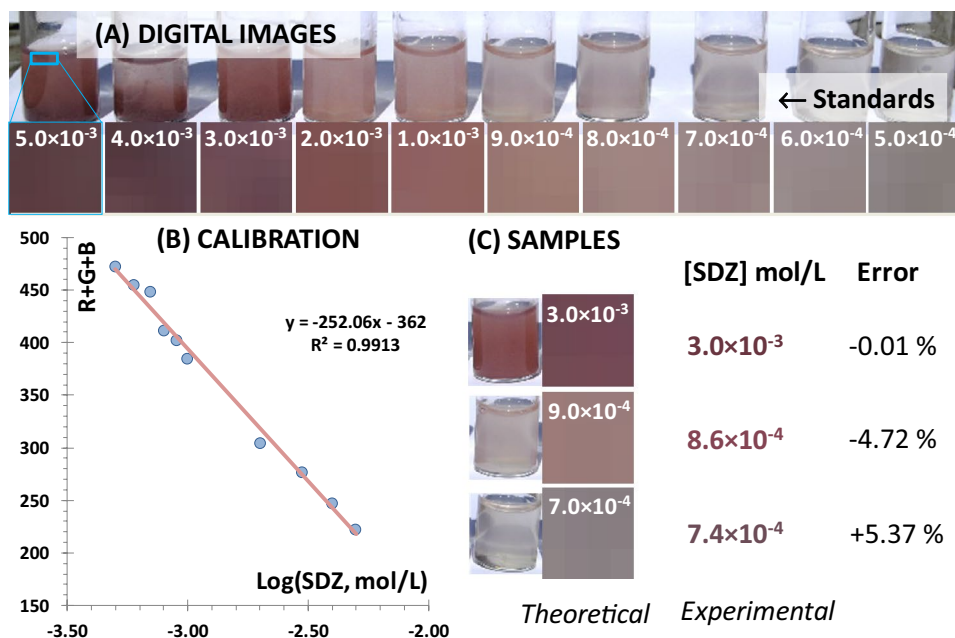


Table 1 Several methods reported for sulfadiazine determination, relying on chemical sensing systems and different transduction schemes

Technique	Recognition system	Linear range	Samples	Refs.
Photoelectro-chemistry	MIP photo-induced sensor with polymethacrylic acid embedded into urchin-shaped Bi ₂ S ₃	0.003–18 mg/L	Water	[49]
Luminescence	MIP modified CdTe quantum dots using 3-aminopropyltriethoxysilane and tetraethyl orthosilicate.	4–20 µM	Seawater	[50]
Luminescence	MIP based in 3-aminopropyltriethoxysilane around quantum dots optosensing material	0.005–1.5 mg/L	Seawater Shrimp	[51]
Luminescence	Quenching effect of SDZ on a MIP material of the fluorescent monomer 7-acryloxy-4-methylcoumarin, with ethylene glycol dimethacrylate and methacrylic acid	1.0–4.0 mM	Milk	[52]
Potentiometry	MIP sensing material on a plasticized PVC membrane casted on a veterinary syringe electrode body evaluated in flow injection analysis system	Down to 0.01 mM	Water	[35]
Potentiometry	Organic porphyrin or cyclodextrin-based ionophores in plasticized PVC membrane	Down to 0.1 mM	Water	[32]

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Compliance with ethical standards

Conflict of interest The author declares that there is no conflict of interest.

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