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Development of paper-based color test-strip for drug detection in aquatic environment: Application to oxytetracycline

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ABSTRACT

The wide use of antibiotics in aquaculture has led to the emergence of resistant microbial species. It should be avoided/minimized by controlling the amount of drug employed in fish farming. For this purpose, the present work proposes test-strip papers aiming at the detection/semi-quantitative determination of organic drugs by visual comparison of color changes, in a similar analytical procedure to that of pH monitoring by universal pH paper. This is done by establishing suitable chemical changes upon cellulose, attributing the paper the ability to react with the organic drug and to produce a color change. Quantitative data is also enabled by taking a picture and applying a suitable mathematical treatment to the color coordinates given by the HSL system used by windows.

As proof of concept, this approach was applied to oxytetracycline (OXY), one of the antibiotics frequently used in aquaculture. A bottom-up modification of paper was established, starting by the reaction of the glucose moieties on the paper with 3-triethoxysilylpropylamine (APTES). The so-formed amine layer allowed binding to a metal ion by coordination chemistry, while the metal ion reacted after with the drug to produce a colored compound. The most suitable metals to carry out such modification were selected by bulk studies, and the several stages of the paper modification were optimized to produce an intense color change against the concentration of the drug. The paper strips were applied to the analysis of spiked environmental water, allowing a quantitative determination for OXY concentrations as low as 30 ng/mL. In general, this work provided a simple, method to screen and discriminate tetracycline drugs, in aquaculture, being a promising tool for local, quick and cheap monitoring of drugs.

1. Introduction

Water is a renewable but finite resource. By 2030, global demands of water will exceed more than 40% the existing resources and more than a third of the world's population will have to deal with water shortages (European Environment Agency (EEA) 2010). Efforts are currently being made throughout Europe towards a reduced and efficient water use and prevention of any further deterioration of the quality of water (European Commission, 2010; European Environment Agency, 2010; European Parliament and of the Council, 2006).

Pharmaceutical drugs are currently among the most common and dangerous organic contaminants, playing a great impact upon the quality of water and biodiversity. Despite its great importance for meeting the needs of the world fish supplies, the aquaculture sector may easily lead to the introduction of drugs in the environment, most importantly in water. A wide range of drugs are employed in intensive fish farming activities, being the use of antibiotics of great concern to public health and environment. The most important classes and groups of antibiotics used in aquaculture include penicillins, cephalosporins, tetracyclines, aminoglycosides, macrolides, glycopeptides, sulfonamides, and quinolones (Kuemmerer, 2009).

Antibiotics are added directly to the water rendering high local concentrations both in water and in adjoining sediments (Cabello, 2006), contributing to increase the total concentration of these drugs in the water and disseminate these throughout the environment (Kuemmerer, 2009). There are three main risks deriving from the presence of antibiotics in the waters: direct organic damage to water; impact on the biotic environment; and lastly, the most feared one, indirect effects on health via resistant microorganisms (Kemper, 2008; Li et al., 2012; Luis Martinez, 2009; Sarmah et al., 2006). The increasing emergence of antibiotic resistance in human pathogens is today a great concern to public health, not only for hindering the successful treatment of infectious diseases, but also for obstructing the combat to other

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pathologies in which antibiotic prophylaxis is needed for avoiding associated infections (Luis Martinez, 2009; World Health Organization, 2000).

Avoiding the environmental impact of antibiotics promoted by fish farming activities is thus fundamental. Considering that antibiotics are essential to maintain fish in healthy conditions for long periods of time under intensive production, the introduction of antibiotics upon the aquatic environment should be set to a minimum. This (apparently) simple target requires a simple measuring process, to enable drug control in multiple stages, after the drug is introduced in water. A test strip material, equipment-free, would meet these requirements.

As far as we know, there are only two colored sensing strips available in the literature for detecting drugs in aquatic environment, both devoted to Quinolone antibiotics (Lara Guerreiro and Ferreira Sales, 2011; Silva et al., 2012). These strips consisted of plasticized PVC entrapping a suitable reagent, with which the antibiotic would form a colored complex. The colorimetric reaction took place in the solid/liquid interface established between the plasticized PVC layer (containing the reagent) and the sample solution (containing the antibiotic). Although the analytical procedure was simple and successful, there are however two aspects of the conception of these strips that could be improved: (1) the supporting material of the strip should consist of renewable material to diminish the environmental impact of an intensive use of such plasticized PVC strips; (2) the complexing reagent should be immobilized in an organized nanostructured assembly (instead of simple entrapment) to allow lowering the detection limit of the test-strip.

Thus, this work proposes the use of cellulose paper as supporting and its subsequent chemical modification by a self-assembly approach to bind a reagent that would sensitize this material to the presence of the antibiotic. This concept was applied herein to Oxitetracycline (OXY), an antibiotic of the tetracycline group. Since the detection/determination of the antibiotic should account a color change to avoid the use of analytical equipment, OXY was made react first with different metals, identifying this way the most suitable colored complex in this context. The metal(s) was then attached to the cellulose paper following suitable nanostructured modification. The optimization and characterization of the final paper test-strip is described, along with the application of the final sensory material. In all cases, the detection/determination of OXY is assessed by visual detection of the color change or mathematical handling of the color coordinates of the images, captured by a normal camera and read in Paint software of Windows.

2. Experimental section

2.1. Materials and reagents

Deionised water < 0.1 μS/cm was employed throughout this work. Chemicals were pro-analysis grade and obtained from different sources: iron (III) chloride ($FeCl_3$) 6-hydrate (Scharlau), lead (II) nitrate (Riedel Haen), copper (II) sulfate 5-hydrate (Panreac), ammonium chloride (Panreac), hydrochloric acid (HCl, Panreac), sodium hydroxide (NaOH, Scharlau), calcium hydroxide (Panreac), magnesium chloride 6-hydrate (Panreac), aluminum sulfate 16-hydrate (BDH), 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES, Sigma), tetracycline (Applichem), zinc chloride (Merck), ethylenediamine (Merck), glutaraldehyde (GLUT, Fluka), chloramphenicol (CRP, Fluka), oxytetracycline (OXY, Fluka), sulfadiazine (SDZ, Fluka), norfloxacin (NOR, Fluka), amonium chloride (NH_4, Merck), disodium hydrogenephosphate (PO_4^{3-}, Sigma Aldrich)and 3-triethoxysilylpropylamine (APTES, Acros). The ethanol used was of 96% and the paper for the test strip was from Fanoia (Reference S-300).

2.2. Apparatus

Spectrophotometric measurements were made in a Thermo Scientific Evolution 201, spectrophotometer. The digital image of the paper test-strips was acquired by a digital camera Samsung PL150, 8 megapixels. The color coordinates of each image was acquired by the HSL color system (Hue, Saturation and Lightness of the HSL space) and measured by using the Paint program of Windows. An ultrasonic bath (Bandelin Sonorex Digitec, model DT 31) and/or a magnetic stirrer (Scansci, MS-H280Pro), were used to promote the dissolution of the solids.

Infrared spectra were collected at a Fourier Transformed Infrared Spectrometer (FTIR, Thermo Scientific Nicolet IS10) equipped with an Attenuated Total Reflectance (ATR) accessory of diamond crystal.

2.3. Metal selection

The colorimetric reaction was tested between OXY and Fe(III), Al(III), Pb(II), Mg(II), Cu(II), Ca(II), NH_4^+ e Zn(II). This was done by mixing 2.0 mL OXY solution 1.0×10^{-3} mol/L with 1.0 mL of an aqueous solution contained the metal species with a concentration of 1.0×10^{-2} mol/L. Acidic and neutral conditions were tested by preparing all solutions in 1.0×10^{-3} mol/L HCl or 1.0×10^{-2} mol/L HEPES. When in the presence of color, the UV/Vis spectrum was recorded to assess the wavelength maximum absorbance and the corresponding absorption intensity.

2.4. Chemical modification of cellulose

The cellulose paper was cut in 1×1 cm^2 peaces and chemically modified by incubating these pieces in different solutions/conditions. The incubation was always conducted under constant stirring (lateral motion), usually at 20 °C (unless specified otherwise), protected from light and ensuring that the paper was completely submerged in the solution. This modification followed the three different stages presented next and was followed by Raman spectroscopy studies (the samples were analyzed directly as modified, not requiring previous sample pre-treating).

2.4.1. Reaction with APTES

The first modification of the paper aimed its amination and was tried out in different ways: (i) incubating the paper in APTES solution prepared in ethanol, in different concentrations (1%, 10%, 50% and 100%), and for up to 3 h; (ii) incubating the paper in sodium periodate solution (0.20 mol/L in water, adjust to pH 4.4 by adding few drops of NaOH 1.0 mol/L) for up to 2 h, proceeded by a second incubation in ethylenediamine solution (2 mol ethylenediamine solution:1 mol sodium periodate solution); (iii) or incubating the paper in potassium persulphate 0.20 mol/L, added of sodium hydroxide 1.0 mol/L until the pH 4.4 was reached, and followed by incubation in ethylenediamine solution, in a similar approach to that indicated previously. The success of these different modifications was followed by completing the modification and incubating, for 5 min, the modified paper in aqueous solutions of OXY with different concentrations (1.0×10^{-2} mol/L, 5.0×10^{-3} mol/L and 1.0×10^{-3} mol/L).

2.4.2. Selection of metal concentration

The cellulose/APTES material was after incubated in solutions of the selected metal of 1.0×10^{-3}, 1.0×10^{-2} and 1.0×10^{-1} mol/L. The produced cellulose/APTES/Metal material