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Novel optical PVC probes for on-site detection/determination of fluoroquinolones in a solid/liquid interface: Application to the determination of Norfloxacin in aquaculture water

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ABSTRACT

A novel optical disposable probe for screening fluoroquinolones in fish farming waters is presented, having Norfloxacin (NFX) as target compound. The colorimetric reaction takes place in the solid/liquid interface consisting of a plasticized PVC layer carrying the colorimetric reagent and the sample solution. NFX solutions dropped on top of this solid-sensory surface provided a colour change from light yellow to dark orange.

Several metals were tested as colorimetric reagents and Fe(III) was selected. The main parameters affecting the obtained colour were assessed and optimised in both liquid and solid phases. The corresponding studies were conducted by visible spectrophotometry and digital image acquisition. The three coordinates of the HSL model system of the collected image (Hue, Saturation and Lightness) were obtained by simple image management (enabled in any computer).

The analytical response of the optimised solid-state optical probe against concentration was tested for several mathematical transformations of the colour coordinates. Linear behaviour was observed for logarithm NFX concentration against Hue+Lightness. Under this condition, the sensor exhibited a limit of detection below 50 μM (corresponding to about 16 mg/mL). Visual inspection also enabled semi-quantitative information. The selectivity was ensured against drugs from other chemical groups than fluoroquinolones.

Finally, similar procedure was used to prepare an array of sensors for NFX, consisting on different metal species. Cu(II), Mn(II) and aluminon were selected for this purpose. The sensor array was used to detect NFX in aquaculture water, without any prior sample manipulation.

1. Introduction

Aquaculture is an important sector worldwide, granting fish meat to future generations at relatively low costs. Its great success comes however with many chemicals disposed throughout the environment, with great danger to finite water resources. This includes antibiotics, fungicidal, pesticides, hormones, anaesthetics, various pigments, minerals and vitamins.

Antibiotics are drugs with the ability to kill or inhibit the growth of microorganisms. Their use in meat producing animals has been controversial. The benefits of their use are obvious, but their abusing use leads to the accumulation of residues in different environmental sections and an increased risk for growth and promotion of bacterial resistance (Picó and Andreu, 2007;Beausse, 2004; Karthikeyan and Meyer, 2006). Furthermore, their wastes are potentially persistent and can be found in fish and water (Beausse, 2004; Karthikeyan and Meyer, 2006; Díaz-Cruz et al., 2003). The first measure to avoid this scenario is to prevent drug spread by having a strict control of the applied doses. This can be established by monitoring the waters from the tanks where the fish are being farmed. This should be done regularly and with a low cost/quick procedure. The drug concentration changes from the time of administration and additional costs in the fish production must be kept to a minimum.

Fluoroquinolones are one of such groups of antibiotics used in fish farming, already correlated to the emergence of resistant bacteria (Hoope, 2001). Many techniques have been employed for their determination in farmed fish and waters. These include the classical microbiological methods (Cabello, 2004; Park et al., 2007) that have the disadvantage of taking too long to produce an analytical result and being subject to biological variability. Separative methods based in liquid-chromatographic techniques coupled to mass-spectrometry (Kassab et al., 2005; Dufresne et al., 2007),

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fluorescence (Zhang et al., 2007; Chui-Shiang et al., 2008) and/or a combination of these (Schneider et al., 2005), and electrophoresis (McCourt et al., 2003) have also been used. These are great in terms of accuracy and robustness, but unable to carry out on-site analysis. Surface Plasmon Resonance and fluorimetric sensors have also been descried (Hueta et al., 1989; Jeon et al., 2008), but they use highly expensive and/or not-portable equipment.

An alternative procedure for field evaluations is a coloured sensing strip, made with low cost materials and allowing easy performance and quick response for a specific target analyte (Guerreiro and Sales, 2011). This is a kind of an optical sensor, where an immobilized reagent must be accessible to interact with the analyte (Wolfbeis, 2008). There are several strategies for reagent immobilization (Khezri et al., 2008; Jeronimo et al., 2007; Narayanaswamy, 2006), with polymeric entrapment by plasticized PVC allowing an easy cast procedure with relatively inexpensive material.

The reagent to immobilize must form a coloured complex with the target analyte. Many colouring agents exist in the literature for fluoroquinolones, including NFX (Kaur et al., 2008; Issopoulos, 1898). These include iron chloride(III), aluminon, cupper sulfate(II) and manganese chloride(II). To produce a useful optical probe, the colouring agent should also have a relative hydrophilic character. It must diffuse from the solid matrix towards the aqueous phase deposited on top of the sensory surface in order to meet the analyte and react with it. Metal species are always a good choice under these circumstances.

Thus, a novel colour disposable probe is presented for on-site detection of NFX. A selected metal is selected among several colouring agents and immobilized on a PVC membrane containing a plasticizer. It is deposited later over a polycarbonate solid support. NFX contacts with the immobilized metal and grants the formation of a coloured product detected by human eye. The same principle was applied to additional colouring agents, allowing multiple responses for the same target analyte.

2. Experimental

2.1. Apparatus and reagents

Spectrophotometric measurements were made in a Thermo® Scientific, Evolution 300, spectrophotometer, carrying a 1 cm glass cell. The digital image of the solid surfaces was acquired by a digital camera Nikon. The Paint program of Windows was used for gaining the coordinates Hue, Saturation and Lightness of the HSL space. This specific colour model was used because it is available in any computer program of windows.

The pH of buffer solutions was measured in a pH Meter GLP 22, Crison®, connected to a combined electrode Consort. An ultrasonic bath, Fungilab SA, and/or a magnetic stirrer, Agimatic-N, were used to promote the dissolution of the solids. Commercial polycarbonate plates (Kartell) with 200 μL wells were used to cast the PVC-based probe.

All chemicals were pro-analysis grade and the ionised-water was employed (conductivity < 0.1 μS/cm). NFX, bis(ethylhexyl)-sebacate (BES), *O*-nitrophenyl octyl ether (oNPOE), di-*n*-octyl phthalate (DOP), dibutyl phthalate (DBP), bis(ethylhexyl)phthalate (BEP), phenyl glycil ether (PGE), poly(vinyl chloride) (PVC) of high molecular weight and manganese chloride were purchased from Fluka. Tetrahydrofuran (THF) was obtained from Panreac. Iron(III) chloride hexahydrate, aluminon and copper(II) chloride were purchased to Merck. Aluminum nitrate, lead(II) nitrate, ammonium molybdate tetrahydrate, nickel nitrate hexahydrate, cobalt(II) nitrate hexahydrate, tin(IV) chloride, vanadium(V), tetracycline, sulfadiazine, chlorpromazine, enrofloxacine, trimethoprim and oxytetracycline were produced by Sigma.

2.2. Metal selection

The colorimetric reaction was tested between NFX and Fe(III), Al(III), Pb(II), Aluminon, Mo(II), Mn(II), Ni(II), Cu(II), Co(II), Sn(IV) e V(V). Acidic and near neutral conditions were tested by preparing all solutions in 0.05 M H_2SO_4 and/or 1.0×10^{-2} M HEPES buffer.

The complexes were investigated by recording the visible spectra of individual and mixed components. Single or mixed solutions of 6.7×10^{-4} M NFX and 3.3×10^{-3} M metal species were used for this purpose.

2.3. Characterization/optimization of the coloured complex

2.3.1. pH effect

The effect of pH on the formation of metal/NFX complex was tested for 1.0×10^{-3} M acetate buffer with variable pHs: 2.4; 3.3; 4.1; 5.0 or 6.1. Metal standard solutions ranged from 1.1×10^{-5} to 2.1×10^{-4} M with a NFX concentration set to 1.0×10^{-4} M. Absorbance was examined at the wavelength of maximum absorbance (λ_{max}) and plotted against concentration.

2.3.2. Molar ratio

Several solutions were prepared with varying amounts of metal (from 2.5×10^{-5} to 2.5×10^{-4} M) and a fixed NFX concentration (1.02×10^{-4} M) in pH 3 acetate buffer. The absorbance readings for λ_{max} were plotted against metal concentration.

2.3.3. Stability within time

The absorbance of several solutions was monitored over 24 h. The concentration of NFX was always 1.03×10^{-4} M, prepared in acetate buffer 1×10^{-3} M, pH 3. Metal concentrations ranged from 3.33×10^{-5} to 6.67×10^{-4} M.

2.3.4. Selection of metal concentration

NFX solutions ranging from 2×10^{-5} to 7×10^{-4} M and were prepared in pH 3.0 buffer and fixed metal concentrations (1.2×10^{-2}, 1.3×10^{-3} or 3.8×10^{-4} M). The absorbance values in λ_{max} were plotted against NFX concentration.

2.3.5. Effect of interfering species

The interfering analysis was conducted for 3.50×10^{-5} M NFX solutions of pH 3.0, carrying an exceeding amount of the previously selected ligand (1.0×10^{-3} M) and an additional divalent metal. Ba(II), Pb(II), Hg(II), Mn(II), Cu(II), Zn(II), Cd(II) and Fe(II) used.

Absorbance was read at λ_{max} of the coloured complex. The degree of interference of each species was the relative error produced in the absorbance signal.

2.4. Solid-state probe

2.4.1. Preparation

Sensory surfaces were prepared by mixing specific amounts of metal, PVC and plasticizer. The mixture was stirred until the PVC was well moistened, and dispersed in 2 mL of THF. A volume of 50 μL was casted on each cavity of a polycarbonate plate and let dry for 30 min.

Variable amounts of the selected metal (0.50, 1.00, 1.50 or 0.25 g) were tested with 0.050 g of PVC, and 0.100 g of plasticizer. Different plasticizers were also employed using only the previously selected amount of metal: BES, oNPOE, DOP, DBP, BEP and PGE. Following, different amounts of plasticizer were tested for the previously chosen plasticizer: 0.02, 0.030, 0.05, 0.10 and 0.15 g. Each condition was tested in at least two independent probes.