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Paper-Based Biosensors for Analysis of Water

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Abstract

The presence of contaminants in water generates a great concern worldwide. As contaminants, we can refer different classes of chemicals, such as pharmaceuticals, personal care products, heavy metals, and also microorganisms, such as waterborne pathogens. Some of the chemical compounds have the potential to bioaccumulate in the aquatic biota. Hence, the development of simple and portable methods for the detection of contaminants in the aquatic environment can improve their monitoring and, consequently, the study of their environmental impact. In this context, the development of paper-based analytical tools and also of biosensor devices has been exploited for quantitative and semiquantitative analysis of several contaminants in different water matrices. The association of these two analytical strategies can provide the implementation of low-cost, portable, and easily handled methods for detecting chemical and biological contaminations in water. In this chapter, we provide a review of the developed paper-based analytical biosensors, highlighting the features of the paper-based (paper substrate and fabrication procedures) and biosensor devices (transducers and biorecognition elements). Moreover, the application of the referred paper-based biosensors for the detection of different water contaminants (pathogens, pharmaceuticals, and heavy metals) in environmental and wastewater samples is discussed.

Keywords: microfluidic, paper-based devices, water analysis, water contaminants, biosensing

1. Introduction

The contamination of the different water compartments with several chemicals and by-products has become a major concern for human health and aquatic biota [1–3]. The environmental contaminants of major concern are pharmaceuticals, personal care products, pesticides and herbicides, heavy metals, and waterborne pathogens. Some of the chemical compounds are persistent to degradation and, therefore, can accumulate in aquatic organisms and sediments. Thus, the monitoring of contaminants in the aquatic environment is crucial to study their impact [4].

Currently, there is no regulation about the allowed levels of pharmaceutical compounds, including ethinylestradiol and antibiotics, in water. Concerning the maximum contaminant levels (MCL) in drinking water for the target metals presented in this work, their MCL are between 2 and 30 $\mu\text{g L}^{-1}$, according to the chemical species [5, 6]. Arsenic presents an MCL value of 10 $\mu\text{g L}^{-1}$ [5, 6], while the MCL value

for mercury is 2 or 6 $\mu\text{g L}^{-1}$ according to United States Environmental Protection Agency (EPA) and World Health Organization (WHO), respectively. Moreover, the MCL for lead is 15 [5] and 10 $\mu\text{g L}^{-1}$ [6]. Uranium presents the higher MCL, which is 30 $\mu\text{g L}^{-1}$ [5, 6]. Cadmium's maximum contaminant level corresponds to 3 and 5 $\mu\text{g L}^{-1}$ according to WHO and EPA, respectively. With respect to the pathogens targeted in the paper-based biosensors, the EPA [7] recommends that *Escherichia coli* cannot exceed 126 CFU per 100 mL in fresh recreational water, while *Enterococcus* should present a maximum of 35 CFU per 100 mL in marine and freshwater.

In this context, paper-based biosensor devices combine the main features of paper substrates (cost-effectiveness, easy manipulation, and compatibility with proteins and biomolecules), with the high specificity and selectivity of the biorecognition systems of biosensors [1, 8, 9]. Furthermore, paper-based assays can be a solution in resource-limited contexts, as both sample and reagents can be introduced without any flow device, through imbibition and filtration via capillary action [10].

The first types of paper-based devices were related to semiquantitative analysis of glucose in urine and immunoassays on chromatographic paper test strips (or lateral flow) [11]. In the last decade, a new fabrication method based on wax patterning was introduced, allowing the design of well-defined channels on paper surface, which provided microfluidic features to the paper-based devices [12].

Reviews concerning the application of paper-based devices in different fields such as food, water analysis, environmental monitoring, and health diagnostics are available [8, 11, 13]. Furthermore, the application of biosensors has been extensively discussed regarding both their usefulness on assessing environmental and urban pollutants [1], and also their role as part of portable biochemical detection systems [14]. However, gathering information about the implementation of paper-based techniques coupled with biosensor devices to water analysis is still lacking. Hence, the aim of this work is to provide a description of the state of the art about the development and application of paper-based analytical biosensors to detect contaminants in water, focusing on work developed in the last 3 years.

2. Paper-based analytical devices

2.1 Substrate material

Paper is a complex material and a promising support for the development of biosensor analytical devices. Its main features, such as versatility, low-cost, and biocompatibility, generate simple and disposable bioanalytical tools using low reagent consumption (in the order of microliters) [8, 11, 12]. Paper is mainly constituted of cellulose fibers. The cellulose is a hydrophilic polymer, which makes paper substrate permeable to aqueous liquids [15].

There are different types of paper that are used for fabrication of paper-based devices (**Table 1**). Filter papers have been widely used as substrate material to paper-based devices for biosensor application [16–19]. There are a vast range of commercially available high-quality filter papers, mainly constituted of alpha cellulose, a highly stable form of cellulose. The filter papers can be classified according to different properties, such as particle retention, pore size, thickness, and flow rate.

The filter paper grade 1, considered as a medium retention and flow filter paper, has been functionalized to obtain paper-based immunosensors [16, 17]. Irvine et al. adsorbed metallothioneins in grade 1 filter paper for the detection of heavy metals [18]. In the same way, other filter paper grades have been used, such as the slow filter paper grade 42 (pore size of 2.5 μm) for the incorporation of an in vitro transcription/translation system [19].

Type of paper	Examples	Features
Cellulose filter paper	Whatman [®] filter paper grades 1 and 2	Hydrophilic polymer, permeable to aqueous liquids; available with different pore sizes and thickness
Cellulose chromatography paper	Whatman [®] chromatographic paper grades 1, and 2	Allows the concentration of nanostructures in its surface and the separation of nanoparticles in agglutination-based assays
Nitrocellulose membrane	Millipore Hi-Flow Plus HF240	Hydrophobic polymer; adequate for immobilization of complex biological structures by electrostatic interactions
Printing paper	Fabriano 5 HP paper, Boise [®] Aspen [®] 30 multiuse recycled copy paper	3D structures can be easily printed in its surface, providing microchannels or screen-printed electrodes

Table 1.
Types and features of papers used in biosensors described recently.

Cellulose chromatography paper is also an alternative as high-quality substrate in paper-based biosensors. These papers can be differentiated by their flow rate and thickness. Vijitvarasan et al. [20] developed a paper-based device taking in advantage of the separative properties of chromatographic paper in order to enhance the concentration of gold nanoparticles (AuNPs) on the surface of the paper. Thus, a lower level of reduced silver particles was detected on the surface of AuNPs. Chromatography papers have also been applied in the development of microfluidic devices based on capillary flow measurement [21, 22]. McCracken et al. [22] tested two different chromatography papers (grade 1 Chr and grade 2 Chr) to optimize the separation of immunoagglutinated particles. The chromatography paper providing the lower flow rate (grade 2, 115 mm/30 min) was selected for the immunoagglutination assay.

Derivatives of cellulose, such as nitrocellulose membranes, have been applied as substrates of paper-based devices. These membranes are naturally hydrophobic and demonstrate to be adequate for the immobilization of enzymes and proteins by electrostatic interactions [11]. For instance, Lopez-Marzo et al. [23] developed a lateral flow immunodevice with nitrocellulose membrane for the detection of Cd²⁺ in water, taking advantage of the immobilization of antibodies (2A81G5 mouse antibody and antiovine serum albumin (BSA) mouse antibody), to create zones where the probe conjugate and the positive control containing BSA would interact. A similar approach was reported for immobilization of BSA conjugate and control goat antimouse immunoglobulin for the detection of U(VI) [24].

Concerning electrochemical paper-based devices, printing paper can be applied in the development of screen-printed electrodes. Hence, carbon-based conductive ink is printed onto paper surface [25, 26]. In this context, Rengaraj et al. [27] fabricated a paper-based electrode with the high-quality printing paper using only three layers of printing.

In addition, both chromatographic paper and multiuse recycled copy paper were used for printing wax wells in paper-based devices as a confinement strategy [20], or as a low-cost alternative to microplates [28].

2.2 Fabrication procedures

There are different techniques that can be applied in order to obtain paper-based biosensor devices with variable properties, such as functionalized platforms with

Figure 1.

Examples of fabrication schemes for production of (A) wax printed paper-based well devices and (B) stencil-printed transparency film-based carbon electrodes. Adapted and reprinted with permission from [28]. Copyright 2017 American Chemical Society.

biomolecules or cell suspensions, conductive characteristics for electrochemical analysis, and create barriers to define the reaction zones.

Wax printing is a process used to create hydrophobic barriers that define reaction microzones or fluid reservoirs [29], as exemplified in **Figure 1**. Different works [16, 17, 20, 22, 28] applied this technique using graphic design software [16, 17, 22] or stencils [28] to define the microchannel areas. The incorporation of the wax onto the microfluidic channel is performed by printing the wax onto the paper surface with subsequent heating to allow wax penetration in the paper.

Screen printing is another technique used to fabricate paper-based devices, particularly for electrochemical analysis (**Figure 1B**). For example, Rengaraj et al. [27] fabricated a paper-based electrode by printing three layers of a carbon-based conductive ink onto hydrophobic printer paper. Other fabrication techniques include a simple procedure of cutting by punching [18, 19], obtaining discs with millimetric dimensions that can be functionalized and/or introduced into devices, such as commercial screen-printed electrodes [18].

Furthermore, lateral flow immune-based devices can be fabricated by assembling different layers, which include the conjugation of pad strip (signal producer), the nitrocellulose membrane, as well as the sample and absorption pad [23, 24]. Stocker et al. [30] applied a simple technique based on premarking the spots with a pencil, with subsequent physical deposition of a cell suspension and drying of the paper strips.

3. Integrated biosensors methods

3.1 Transducers

Biosensors can be defined as analytical devices, which integrate or associate a biorecognition element and a transducer. The bioelement recognizes the target analyte and the transducer converts the biochemical interaction to a measurable signal [1]. The most frequently applied transducers are based on optical, electrochemical, thermal, and piezoelectric properties. This work focused on the

transducer types most used in the paper-based biosensors for analysis of water (electrochemical, optical, and piezoelectric).

The electrochemical paper-based biosensors are based on the modification of paper-based platforms placed in commercial screen-printed electrodes [17, 18], or rely on the fabrication of lab-made functionalized paper-based screen-printed electrodes [27].

Concerning the optical approaches used in the paper-based devices, the most applied strategies were based on colorimetric measurement [19, 20, 28] using image processing algorithms as an analytical system. In this context, a colorimetric based approach for the detection of Pb^{2+} and for U(VI) resorted to the acquisition of images of the paper spots with a digital camera, and images processed using the ImageJ software [20, 24]. On the other hand, Adkins et al. [28] developed a paper-based colorimetric method for the detection of *Escherichia coli*, which was based on a smartphone for image acquisition and ImageJ software for image processing.

Other example of mobile-based strategy involves the quantification of different pathogens (*E. coli* and Zika virus) as a function of capillary flow rate using a smartphone as a photometric detector [21]. This photometric approach was based on video recording of an immunoassay followed by comparison of the capillary flows between different analyte concentrations. Other colorimetric method was based on the detection of Cd^{2+} [23] in drinking water with a lateral flow immunosensor device. The measurement of color intensity was performed with COZART™ RapidScan color intensity portable reader. Colorimetry in paper-based biosensor devices was developed as a semiquantitative approach for the detection of arsenite [30]. This method was based on a bacterial biosensor deposited onto a paper strip. The developed color measurement was performed by comparison with spots containing known arsenite concentrations. Furthermore, a method based on fluorescence was applied as transducer for the detection of ethinylestradiol in a paper-based immunoassay [16]. For this, a LED-based system was constructed and used as excitation source. The fluorescence emission was measured with a scientific-grade spectrometer.

A piezoelectric strategy was also implemented for measurement of immunoagglutinated samples for the detection of *E. coli* and Zika virus [22]. This approach was based on particle rheology of the immunoagglutinated samples. In order to monitor the movement of the suspension of particles in a microfluidic paper-based platform, videos were taken with a smartphone and flow distance was measured every five frames.

3.2 Biorecognition elements

The biorecognition element has a strong and selective affinity to the target. There are several types of biorecognition elements, such as natural biomolecules (nucleic acids, antibodies, enzymes, and other proteins), synthetic bioelements (molecularly imprinted polymers, aptamers), or whole cells (**Figure 2**) [1].

Different types of biorecognition elements have been applied for the development of paper-based biosensors for the detection of target analytes in water. An in vitro transcription/translation system reconstituted from purified recombinant components necessary for *E. coli* translation of β -galactosidase enzyme was immobilized on paper as a turn on/turn off switcher for the presence of antibiotics inhibiting bacterial protein synthesis [19].

Concerning the application of antibodies as biorecognition elements, specific antibodies (polyclonal rabbit anti-EE2) have been applied for the detection of the estrogen ethinylestradiol in river water samples [16, 17]. In addition, suspensions of antibody-conjugated particles were used for the detection of two target pathogens (*E. coli* K12 and Zika virus) [21]. For the detection of U(VI), immobilized U(VI)-2,9-dicarboxyl-1,10-phenanthroline-BSA conjugate worked as a competitive probe

Figure 2.
Examples of biorecognition elements found in paper-based biosensors.

for the antibody 12F6-AuNP conjugate, as the antibody 12F6 has an increased affinity to U(VI)-2,9-dicarboxyl-1,10-phenanthroline complex [24].

Biomolecules aiming the detection of toxic metals can also be referred. A complex comprising magnetic beads, gold nanoparticles (AuNPs), and the functional nucleotide GR5-DNAzyme was applied as a biorecognition element of lead ion [20]. In another work, the recombinant human metallothionein 1a, a metal-binding protein [18], was used for the recognition of As^{3+} and Hg^{2+} in water. The tetrameric protein lectin concanavalin A (obtained from *Canavalia ensiformis*), selective to carbohydrates on bacterial cells, was selected as a biorecognition element of bacterial cultures from sewage sludge [27].

Finally, whole-cell living bacterial biosensors for arsenite detection were based on genetically engineered *E. coli*, where the *ars* operon (set of structural and regulatory genes whose expression is controlled through arsenite binding) was modified with a sequence for expression of β -galactosidase as a reporter protein in the presence of the target analyte [30]. Whole cells (biofilm formed from anaerobic sludge) were also employed in the biosensor proposed by Chouler et al. [25] for the assessment

of toxic compounds in water in a microbial fuel cell device. The detection principle was based on the conversion of the chemical energy contained in organic matter into electricity via the metabolic processes of microorganisms. Hence, a microbial biofilm is placed on the anode surface, where the electroactive bacteria mediate the transference of electrons to the electrode upon their metabolic activity. Any factor disrupting this (water pollution for instance) will disrupt this signal. A similar approach was proposed by Xu et al. [26] using a wastewater bacteria consortium.

4. Applications

In this section, the application of paper-based biosensors for the detection of different types of target analytes in water samples is discussed. In **Table 2**, the main features of the target analyte, the sample type, the paper substrate, and the fabrication method of the paper-based device, the method of detection, and the biorecognition element are summarized.

Pharmaceuticals are among the targets. Indeed, they are considered emerging environmental contaminants, as they can be harmful to human health and to aquatic life. In this context, the synthetic hormone—ethinylestradiol, one of the main compounds of oral contraceptives, is considered an emerging pollutant due to its potential high estrogenic effect on the biota. Scala-Benuzzi et al. developed two different methods for the detection of ethinylestradiol in river water samples using an antiethinylestradiol specific antibody [16, 17]. In both approaches, the water samples were filtered, and pH was adjusted to 7.0 with phosphate buffer before the analysis. In one work, a fluorescent paper-based biosensor was implemented [16]. This methodology presented a limit of detection (LOD) of 0.05 ng L^{-1} , which is mainly related to the high sensitivity of fluorescence methods. In another work, ethinylestradiol was detected in river water with a paper-based immunosensor based on electrochemical analysis [17], which also reached a low LOD value of 0.1 ng L^{-1} , suitable for environmental analysis.

Antibiotics are another group of pollutants of great concern due to the global threat of antimicrobial resistance and the excessive, and sometimes abusive, use of these compounds. A colorimetric biosensor for screening of several antibiotics (paromomycin, tetracycline, chloramphenicol, and erythromycin) inhibiting bacterial protein synthesis was applied for the detection of antibiotics in surface water [19]. The method was based on the ability of these antimicrobials to inhibit β -galactosidase synthesis. When a water sample without the target antibiotics was placed in the paper-based device, the enzyme β -galactosidase was synthesized and its activity induced a color change on the paper disc surface. However, when antibiotics were present, the inhibition of β -galactosidase synthesis prevented the change of color. Despite the limit of detection was on the microgram per milliliter level (0.5 , 2.1 , 0.8 , and $6.1 \mu\text{g mL}^{-1}$ for paromomycin, tetracycline, chloramphenicol, and erythromycin, respectively), this biosensor can be applied as a simple and portable screening methodology.

Heavy metals are naturally present in the environment. However, these elements can be toxic to human and aquatic organisms even at low concentrations. Moreover, their presence can be increased by industrial and agriculture activities. Vijitvarasan et al. implemented a paper-based scanometric biosensor for the detection of lead in water [20]. The biosensor was applied to river water samples. These samples were filtered, diluted 10 times with 10 mM tris-acetate buffer and spiked with different Pb^{2+} concentrations before analysis. An LOD value of 0.9 nM was determined. Furthermore, a method using a sensitive gold nanoparticle-based lateral flow immunodevice [23] was applied for the quantification of cadmium. Drinking water

Target	Sample type	Paper substrate; fabrication method	Detection method	Biorecognition element	LOD	Reference
Pharmaceuticals						
Ethinylestradiol (EE2)	River water	Filter paper grade 1; wax printing	F	Antibody (anti-EE2)	0.05 ng L ⁻¹	[16]
EE2	River water	Filter paper grade 1; wax printing	E	Antibody (anti-EE2)	0.1 ng L ⁻¹	[17]
Antibiotics inhibiting protein synthesis ^a	Surface water	Filter paper discs, 1442-055; cutting by punching	C	Enzyme (β -galactosidase)	0.5–6.1 μ g L ⁻¹	[19]
Metals						
Arsenic, mercury	Ultrapure water	Filter paper grade 1; cutting by punching	E	Recombinant human metallothionein 1a	13 ppb (As ³⁺); 45 ppb (Hg ²⁺)	[18]
Lead ion	River water; synthetic urine	Chromatography paper; wax printing	C	GR5-DNAzyme	0.3 nM	[20]
Uranium (VI)	River water	Cellulose and nitrocellulose membranes; assembling of different layers in a plastic backing card	C	12F6 antibody against U(VI)-chelator complex	36 nM	[20, 24]
Arsenite	Ground-water	0.5 $\times$ 4 cm paper; cutting	C	<i>E. coli</i>	8 μ g L ⁻¹	[30]
Cd ²⁺	Drinking water, tap water	Hi-Flow Plus nitrocellulose membrane; assembling of different layers in a plastic backing card	C	Cd-EDTA-BSA-AuNP	0.1 ppb	[23]
Pathogens						
<i>E. coli</i> and Zika virus	Deionized water; serum simulant	Paper Chr. grade 2; wax printing	P	Antibody-conjugated particles	1 log CFU mL ⁻¹ ^b ; 20 pg mL ⁻¹ ^c	[21]
<i>E. coli</i> K12 and Zika virus	Deionized water; simulated serum	Paper Chr. grade 2; wax printing	R	Antibody (goat polyclonal)	2 log CFU mL ⁻¹ ^b ; 0.531 ^d	[22]
Bacterial cultures from sewage sludge	Synthetic wastewater	Cotton-based paper; screen printing	E	Lectin concanavalin A	1.9 $\times$ 10 ³ CFU mL ⁻¹	[27]

Target	Sample type	Paper substrate; fabrication method	Detection method	Biorecognition element	LOD	Reference
E. coli, <i>Enterococcus</i> spp.	Lagoon water, alfalfa sprout	Multiuse recycled copy paper; wax printing	C	Substrates ONP and PNP	81 μ M (ONP), 119 μ M (PNP)	[28]
Others						
Water toxicity	Artificial wastewater	Cotton-based paper; screen printing	E	Biofilm formed from anaerobic sludge	0.1 ^e	[25]
Shock pollution	Wastewater	Filter paper; ink coating	E	Bacteria consortium	0.022 ^f	[26]

F, fluorescence; *E*, electrochemical; *C*, colorimetric; *P*, photometric; *R*, rheology-based measurement. PNP: *p*-nitrophenol; ONP: *o*-nitrophenol.

^aParomomycin, tetracycline, chloramphenicol, and erythromycin.

^bLOD value for *E. coli*.

^cLOD value for Zika virus.

^dLOD value for Zika virus expressed as transcription copies mL⁻¹.

^eFormaldehyde concentration monitored (% v/v).

^fPower output slope for Cr (VI).

Table 2.
Paper-based biosensors for analysis of pharmaceuticals, heavy metals, and waterborne pathogens in water.

samples were spiked with 10 and 100 ppb of Cd^{2+} , and other 11 metals commonly found in such type of water, containing also EDTA and ovalbumin (masking agent). An LOD of 0.1 ppb was achieved.

A semiquantitative approach based on a paper-based bacterial biosensor was applied [30] for the detection of arsenite in groundwater samples. It was observed that arsenite produced a visible blue color from substrate of β -galactosidase (reporter protein) at arsenite concentration above $8 \mu\text{g L}^{-1}$. Finally, a paper-based lateral flow device was developed for uranium (VI) determination with an LOD (36 nM) below the action level established by the World Health Organization (126 nM) using an immunological competitive approach [24]. These sensors are a suitable tool for field analysis, in opposition to conventional time-consuming and expensive techniques performed under lab environment.

Sensors based on microbial metabolism were developed for application in wastewaters. Pollution peaks, meaning the abrupt change in concentration of organic and metal pollutant in wastewater treatment plants, can compromise the biological treatment phases by killing or inhibiting microorganisms present in sludge. Hence, untargeted sensors were developed using either biofilms [25] or bacteria consortium [26] to report spiking pollution in wastewater influents.

Waterborne pathogens are a major public health as they can lead to several diseases such as cholera, typhoid fever, and dysentery. Hence, accessible, cheap, and disposable analytical tools for monitoring the presence of these pathogens are mandatory, especially in areas with low resources. In this context, an electrochemical paper-based biosensor [28] was developed for the detection of *E. coli* in water, as an indicator of fecal contamination and an indirect indicator of the presumptive presence of other gastrointestinal bacteria. Different *E. coli* strains (both pathogenic and nonpathogenic) were detected in uninoculated and inoculated lagoon water. The method was able to detect as low as 10 CFU mL^{-1} of pathogenic and nonpathogenic *E. coli*.

5. Conclusions

The paper-based biosensors developed for quantification of the synthetic hormone EE2 presented higher sensitivity when compared to the more complex and

Figure 3. Schematic representation of the elements that compose paper-based biosensors.

expensive LC-MS/MS methods [31, 32]. With respect to the developed biosensors for quantification of different metals (uranium [24], arsenite [30], and cadmium [23]), the LOD was lower than their respective MCLs, thus complying with regulatory requirements. Furthermore, *E. coli* was detected [21, 22] at concentration level similar or lower than the maximum CFU/mL allowed in fresh recreational water. Hence, the use of paper-based platforms in biosensors has allowed the development of simple, specific, sensitive, and portable devices for the detection of several types of target analytes in water, with possible features summarized in **Figure 3**. Most of the reported methods were applied to surface water and drinking water samples only, which are samples containing a reduced amount of organic matter when compared to wastewater. Hence, efforts to develop sensors that can deal with more complex matrices should be pursued, encompassing strategies that accommodate sample pretreatment.

The most frequently used transducers comprised electrochemical and optical methods, with analytical strategies based on colorimetric reactions, associated with image processing analysis. Besides the recent advances in the development of paper-based analytical tools and biosensor devices, their association to the analysis of contaminants in water is still an open research field with a high potential for the implementation of new portable and low-cost analytical methods for *in situ* analysis.

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Conflict of interest

We declare that there is no conflict of interest.

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