

Contents lists available at ScienceDirect

## **Biosensors and Bioelectronics**



journal homepage: www.elsevier.com/locate/bios

# Ultrasensitive immunochromatographic assay for the simultaneous detection of five chemicals in drinking water



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#### ARTICLE INFO

Article history: Received 19 September 2014 Received in revised form 17 November 2014 Accepted 1 December 2014 Available online 3 December 2014

Keywords: Gold nanoparticle immunochromatographic strip Simultaneous detection Microcystin-leucine-arginine Chloramphenicol Testosterone

#### ABSTRACT

In this paper, we describe the development of a multicomponent lateral-flow assay based on an antibody-antigen reaction for the rapid and simultaneous detection of trace contaminants in water, including a heavy metal, algal toxin, antibiotic, hormone, and pesticide. The representative analytes chosen for the study were lead (Pb(II), microcystin–leucine–arginine (MC–LR), chloramphenicol (CAP), testosterone (T), and chlorothalonil (CTN). Five different antigens were immobilized separately in five test lines on a nitrocellulose membrane. The monoclonal antibodies specifically recognized the corresponding antigens, and there was no cross-reactivity between the antibodies in the detection assay. Samples or standards containing the five analytes were preincubated with the freeze-dried colloidal-gold-labeled monoclonal antibody conjugates to improve the sensitivity of the assay. The results were obtained within 20 min with a paper-based sensor. The cut-off values for the strip test were 4 ng/mL for Pb(II), 1 ng/mL for MC–LR, 0.1 ng/mL for CAP, 5 ng/mL for T, and 5 ng/mL for CTN. The assay was evaluated using spiked water samples, and the accuracy and reproducibility of the results were good. In summary, this lateralflow device provides an effective and rapid method for the onsite detection of multiple contaminants in water samples, with no treatment or devices required.

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#### 1. Introduction

Organic contaminants and inorganic pollutants in surface water pose a risk to water quality and the health of ecosystems throughout the world (Nikolaou et al., 2007; Jones et al., 2002; Subedi et al., 2012; Xu et al., 2009; Golet et al., 2002; Tong et al., 2011). Recent studies of water pollution have showed that the most pervasive contamination is found in China (Liang et al., 2013; Wu et al., 2011). Pharmaceuticals and personal-care products (PPCPs), including prescription drugs, over-the-counter preparations (antibiotics, anti-inflammatory drugs, sedatives, and eikonogen), and cosmetics (heavy metals), are detected in surface water, groundwater, rivers, lakes, soil, and food (Wang et al., 2014; Bu et al., 2013). The most common pharmaceutical compounds in the environment are antibiotics, anti-inflammatory drugs, lipid regulators, cancer therapeutic drugs, steroids, and related hormones (testosterone) (Nikolaou et al., 2007; Richardson and Ternes, 2011; Richardson and Ternes, 2014).

These chemicals are widely used and released into the environment and may have toxic effects on living organisms,

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http://dx.doi.org/10.1016/j.bios.2014.12.004 0956-5663/© 2014 Elsevier B.V. All rights reserved. including humans (Subedi et al., 2012). To assess the risks of these small molecules in drinking water or biological samples, we have developed an efficient, accurate, and simple method to simultaneously detect trace concentrations of small molecules in water samples. In this study, five analytes were used as model targets and were simultaneously detected with a strip sensor: lead (Pb (II)), microcystin–leucine–arginine (MC–LR), chloramphenicol (CAP), testosterone (T), and chlorothalonil (CTN).

Lead is a naturally occurring element that is toxic to both humans and animals, although it has some beneficial uses. Lead accumulates in our bodies over time and can reduce kidney function, produce anemia, and slow growth. Lead can cross the placental barrier, reducing fetal growth and causing premature birth (Edwards et al., 2009).

To prevent the potential health effects of long-term exposure to lead, the National Primary Drinking Water Regulations of China have set the maximum concentration level to 15 ng/mL. Because lead is a persistent and toxic environmental contaminant, a simple and sensitive method must be developed for its detection in drinking water.

MC–LR produced by cyanobacteria is the most toxic naturally occurring toxin (Graham et al., 2010). Water ecosystems are severely contaminated with cyanobacteria in China (Srivastava et al., 2013). The contamination of drinking water or surface water

presents a significant hazard for human health, causing hepatic necrosis and hemorrhage. To ensure water safety, the recommended limit for MC–LR in drinking water is 1 ng/mL.

Chloramphenicol is extensively used in veterinary practice as a broad-spectrum antibiotic against a variety of pathogens. Its systemic use or topical application can cause fatal adverse effects in humans, including aplastic anemia (Page., 1991). The use of CAP in food-producing animals was specifically prohibited in the United States and the European Union (EU) in 1994 (Stolker and Brinkman, 2005). However, the illegal use of CAP causes the pollution of rivers and ultimately the drinking water supplies (Wang et al., 2014). Testosterone is a steroid hormone of the androgen group. Although it can be beneficial to health, the abuse of T is prevalent in today's society. It is administered to animals at poultry farms and fishing grounds to improve their growth rates. However, T disrupts normal physiological functions and interferes with fertility processes. It also migrates through the environment, reducing water quality and damaging environmental and human health (Kolpin et al., 2002).

Chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile) is the third most commonly used fungicide in the USA and has been detected in ambient air and groundwater. The national standards of China limit the level of CTN in drinking water to 10 ng/mL (GB 5749–2006). Chlorothalonil may be a carcinogen in humans because it has been showed to cause kidney cancer in animals. Long exposure causes nose bleeds and skin rashes (Murphy and Haith, 2006).

### These analytes probably occur simultaneously in environmental water and pose a hazard to human health. Many methods have been developed to detect these analytes so that their contamination of environmental water can be monitored. Although liquid chromatography-mass spectrometry (LC-MS) and inductively coupled plasma (ICP)-MS can detect many analogs of these compounds or chemically similar analytes, no instrumental method for the simultaneous determination of Pb(II), MC-LR, CAP, T, and CTN have been developed because their properties differ too markedly. Instrumental methods, such LC-MS and ICP-MS, have been used to detect one or two of them (Li et al., 2006; Neffling et al., 2009; Sheridan et al., 2008; Mattern et al., 1991; Singh, 2008). These methods have the advantage of accurate quantification and have been widely used as reference methods. However, they are costly and time-consuming, and expensive instruments and skilled operators are required. The special instruments required for these detection processes make such methods unsuitable for the on-site and simultaneous detection of various contaminants. The wide occurrence of PPCPs requires rapid and cost-effective methods for the simultaneous detection of multiple analytes. As showed in Table 1, many analytical methods have been used to detect multianalytes, including disease biomarkers, small molecules, oligonucleotide targets, biological thiol molecules, proteins, heavy metals, and mycotoxins, in single samples. In Table 1, the advances in multiplex detection by different analytical methods were listed including Raman scattering technology,

#### Table 1

Advances in in multiplex detections.

Analytical methods	Analytes	
Raman scattering	Intrinsic cancer biomarkers Small molecules Oligonucleotide targets	Dinish et al. (2014) Cecchini et al. (2013) Cao et al. (2002)
Fluorescent emission	Small molecules Biological thiols molecules Multiprotein interactions Small molecules	Nakano et al. (2013) Yang et al. (2014), Liu et al. (2014a,b,c,d) Galperin et al. (2004) Liu et al. (2007)
Spectrally encoded beads	Proteins	Wilson et al. (2007) Han et al. (2001)
Immunoprecipitation	Colon cancer biomarker candidates	Lin et al. (2013)
Electrochemical detection	Heavy metals Heavy metals Heavy metals Heavy metals	Lin et al. (2011) Xu et al. (2014a,b) Rattanarat et al. (2014) Cho et al. (2012)
Multiplex ELISA	Graft versus host disease (GVHD) biomarkers Disease biomarkers Human tumor markers Human tumor markers	Frampton et al. (2014) Rissin et al. (2010) de la Rica and Stevens (2012) Gaster et al. (2009)
Microchip platform	Type 1 diabetes (T1D) markers Liver fibrosis markers A panel of molecules Mycotoxin	Zhang et al. (2014a,b) Shi et al. (2007) Das et al. (2012) Wang et al. (2012)
Array test strip	Heavy metals	Liu and Lin (2014)
Immunochromatographic assay	Mycotoxin Mycotoxin Sulfonamides A panel of molecules	Wang et al. (2013a,b) Li et al. (2013) Guo et al. (2010) Our method

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