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OC18

In-situ monitoring of nitrate levels in water samples using a specially designed microfluidic paper-based device

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One of the main goals of the World Health Organization (WHO) is that "all people have the right to have access to an adequate supply of safe drinking water". Water from wells can be contaminated by a variety of toxic substances, such as pesticides, agricultural and industrial activities, animal waste, or sewage, through surface water runoff. Early detection of well contamination is crucial to prevent harm to public health. Nitrogen is a very important nutrient that is usually present in the nitrate form, but when not absorbed, can accumulate and become a pollutant. Fertilizers is one of the main sources of nitrate in groundwaters. To protect human health and the environment, the Groundwater and Drinking Water Directives establish the maximum allowable concentration for nitrate at 50 mg/L reenforcing the importance of monitorization [1].

Microfluidic paper-based analytical devices, also known as µPADs, are simple, easy-to-use devices that provide rapid and accurate measurements. Furthermore, because they are typically small and don't require complex equipment or external power, they are an ideal tool for in situ monitoring.

In this context the PHsense project aims to design, develop, and study novel microfluidic analytical devices for on-hand public health biomarkers assessment in natural waters and biological fluids of non-invasive collection, in a One Health approach. The idea is to produce fast-response and accurate sensor devices to enable reliable and on-time results, making possible effective public health monitoring. On-hand analytical devices represent a vital strategy for attaining effective monitoring and frequent analysis. However, if a fast chemical/biochemical response tool results in a faster analytical decision, these new tools used for monitoring must be as accurate and effective as possible. According to the ASSURED criteria established by WHO, these new tools must be "affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and derivable to end-users".

This work, within the framework of the PHsense project, aims to address the quantification of nitrate in well water samples using a specially designed µPAD to attain a user-friendly, in the moment and on-site analysis. The nitrate conversion to nitrite is performed by the enzymatic reaction of nitrate reductase and the resulting nitrite is detected by performing the well-known Griess reaction [2]. To effectively use the enzymatic reaction in a microfluidic paper device, a hydrophilic membrane was incorporated in the device. The small pore size of this membrane allows a delay in the vertical flow and consequently enabling a more significant extent of the enzymatic reaction.

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[1] European Environment Agency, Nitrate in groundwater in Europe, published 10 Nov 2025, https://www.eea.europa.eu/en/analysis/indicators/nitrate-in-groundwater-8th-eap, accessed on 13/01/2025.
[2] Ferreira, F. T. S. M.; Mesquita, R. B. R.; Rangel, A. O. S. S. *Molecules* **2021,** *26*, 6355.