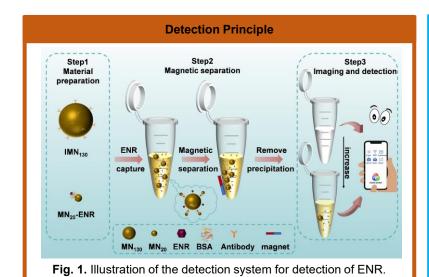


A washing-less biosensor based on the dual functions of magnetic separation and signal output of magnetic nanoparticles for the rapid and visual detection of enrofloxacin

Jiangqi Wang, Yafang Shen, Xingyue Tang, Haiqi Zhang, Guijie Hao *
Key Laboratory of Healthy Freshwater Aquaculture, Ministry of Agriculture and Rural Affairs; Huzhou
Key Laboratory of Aquatic Product Quality Improvement and Processing Technology, Zhejiang Institute
of Freshwater Fisheries, Huzhou 313001, Zhejiang, China

Abstract

There is an urgent need for in-situ detection of antibiotic residues to enhance both food safety and environmental sustainability. Although various biosensing methods have been elaborately designed and achieved extremely high sensitivities, they are always complicated and require sophisticated instruments, making them unsuitable for on-site application. Herein, a simple and washing-less biosensor that took full advantage of magnetic nanoparticles (MNs), i.e., magnetic separation and signal output, was developed for the rapid and sensitive detection of enrofloxacin (ENR) residues. The surface of MNs with diameters of approximately 20nm (MN₂₀) and 130nm (MN₁₃₀) were modified with ENR and antibodies to prepare MN₂₀-ENR and immunomagnetic nanoparticles (IMN₁₃₀), respectively. In the absence of ENR, the MN₂₀-ENR analogues were captured on the surface of IMN₁₃₀, forming "MN₂₀-ENR-IMN₁₃₀" complexes, which were larger in volume and could be quickly separated in a magnetic field. While in the presence of ENR, the target ENR competed for the binding sites on IMN₁₃₀ surface, inhibiting the formation of the "MN₂₀-ENR-IMN₁₃₀" structures. Therefore, the separation speed significantly slowed down. Qualitative or semi- quantitative determination of ENR residues could be achieved via visual observation of the color changes, while quantitative detection was also achieved with the aid of a microplate reader or merely a smartphone. The developed method enabled sensitive, selective and rapid detection of ENR with a limit of detection of 0.18 ng mL⁻¹ and a detection time of 25 min. This method uses MNs for both magnetic separation and signal output, simplifies the detection process and eliminates the use of sophisticated instruments, providing a powerful tool for the rapid and in-situ detection ENR residues in various fields such as food safety and environmental protection.



Results and Discussion

A

Detection (OD₄₆₅)

Remove precipitation Precipitation (OD₄₆₅)

Remove precipitation Precipitation (OD₄₆₅)

Remove precipitation (OD₄₆₅)

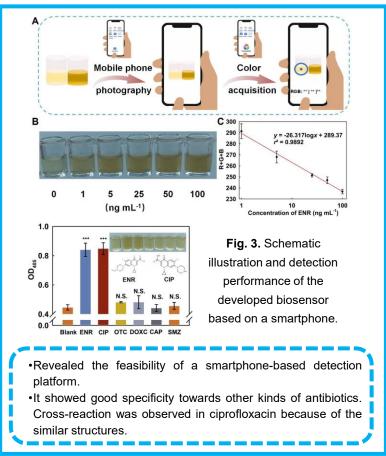
Remove precipitation of ENR (ng mL⁻¹)

The precipitation of ENR (ng mL⁻¹)

Fig. 2. Illustration of the detection process, calibration curve for

•The developed biosensing method exhibited LODs of 0.18 and 10.45 ng mL⁻¹ in PBS and fish extracts, respectively.

the detection of ENR and the linear detection range.



Conclusions

- A simple and washing-less biosensor that took full advantage of magnetic nanoparticles was developed for the rapid and sensitive detection of ENR.
- •The biosensor showed high detection performance towards ENR.
- •This technology could realize simple, fast, and in-field detection of ENR in *M. rosenbergii*.

Acknowledgement

This work was funded by the Public Welfare Technology Application Research Program of Huzhou (2022GZ25) and Zhejiang Special Project for Provincial Research Institutes (2024YSZX01).