Localized surface plasmon resonance biosensor based on polydopamine molecular imprinted polymer for detection of multi-antibiotics in chicken meat

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Abstract

Antibiotics have been widely used in animal food industry since the early 1940s, but have come under consumer criticism due to the potential for antibiotic residues and the development of bacterial resistance. Different countries or organizations have established various maximum residue limits as acceptable levels, and therefore, analytical methods with a low limit of detection (LOD) are critical for identification and quantification of antibiotic residuals for the stewardship of antibiotics. The objective of this project is to develop a localized surface plasmon resonance (LSPR) biosensing system for rapid, sensitive and selective detection of multi-antibiotics in chicken meat, using polydopamine molecular imprinted polymer (PDA-MIP) as the recognition element. Detection targets of enrofloxacin, tetracycline and phthalic acid were used as templates, and the PDA-MIP film was fabricated by polymerization of dopamine and purified powder of target antibiotics in Tris buffered saline on a LSPR sensor chip. After removal of templates, the modified LSPR/PDA-MIP biosensor was used for detection of each target analyte at 6 concentrations in the range of 0 to 1000 ng/mL. In order to amplify the detection signal, competitors conjugated with bovine serum albumin were injected and adsorbed by the residual binding sites on the PDA-MIP film. With amplification, the proposed method allowed a detection time of 20 min and LODs of 66.3, 3.7 and 71.7 ng/mL for target analytes, respectively. During the optimization process, parameters of template concentration was adjusted to increase the sensitivity of detection. The developed LSPR/PDA-MIP biosensing method reduced the detection time compared with most reported analytical methods and showed high potential for rapid and sensitive in-field detection of multi-antibiotic residues.

Introduction

Antibiotic overuse in animal-derived food products would lead to the development of resistant bacterial strains, especially with the use of highly important or critically important antimicrobial groups for human health. Current HPLC and ELISA methods have their limitations for rapid and in-field detection of antibiotic residues.

Localized surface plasmon resonance (LSPR) sensors have shown great potential in biosensing detection studies, due to its reproducible, label-free and real-time features. For detection of antibiotics, published studies commonly use antibodies as the recognition element, however, the instability of antibodies under room temperature makes these methods unsuitable for in-field detection, and therefore the molecularly imprinted method was introduced to develop a potential recognition element for sensitive biosensing detection.

Objective

The objective of this project is to develop a localized surface plasmon resonance (LSPR) biosensing system for rapid, sensitive and selective detection of multi-antibiotics in chicken meat, using polydopamine molecular imprinted polymer (PDA-MIP) as the recognition element.

Materials & Methods

Materials:

- Target analytes:
  - Enrofloxacin (ENRO)
  - Tetracycline (TETR)
  - Phthalic acid (PHTH)
- For preparation of PDA-MIP film:
  - Dopamine (DA)
  - Tris buffered saline, 50 mM, pH 8.0
- For preparation of BSA-ENRO conjugates:
  - Bovine serum albumin (BSA)
  - *N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (EDC)
  - *N*-hydroxysuccinimide (NHS)
  - Dimethyformamide (DMF), anhydrous
- Running buffer:
  - Phosphate buffered saline (PBS), 10 mM, pH 7.4
- Regeneration agent:
  - Sodium dodecyl sulphate (SDS), 0.5%

Apparatus:

- Nicoya two-channel OpenSPR with control and analysis software of NicoyaLifesciences and TraceDrawer

Methods:

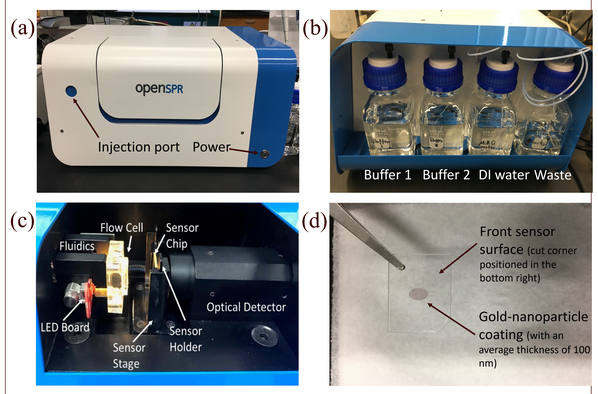
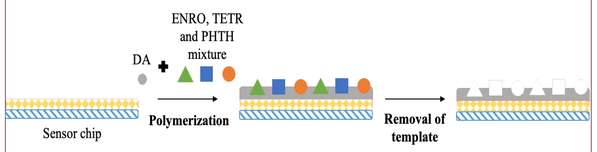
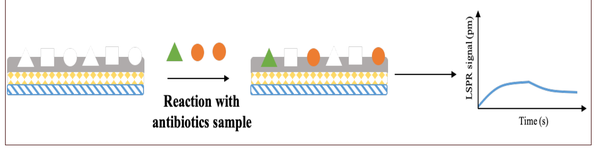


Fig 1. Nicoya two-channel OpenSPR (a) Injection port and powder button; (b) External fluidic setup; (c) Optical and fluidic systems; (d) Sensor chip

Part I. Fabrication of PDA-MIP film



Part II. Direct detection of antibiotics with LSPR



Part III. LSPR signal amplification with competitive assay

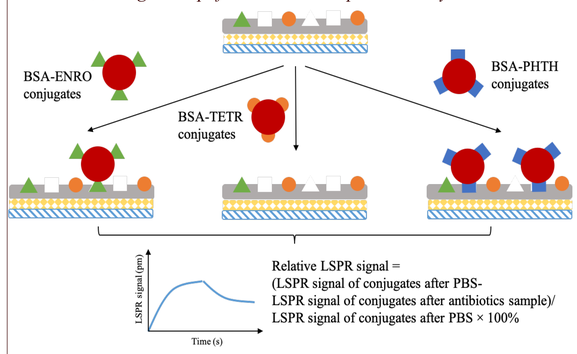


Fig 2. The principle of PDA-MIP film synthesis, BSA conjugate competitor fabrication and real-time direct and competitive detection of ENRO, TETR and PHTH with LSPR

Results

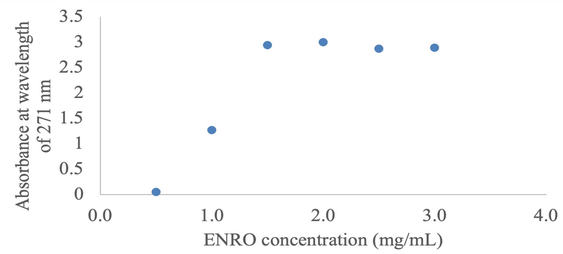


Fig 3. UV-vis spectrophotometry result for the PDA imprinting process optimization with variable as template ENRO concentration

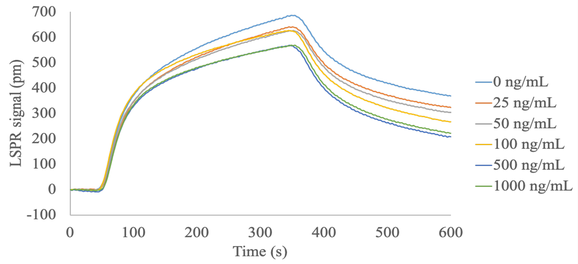


Fig 4. Concentration dependent real-time LSPR sensorgram of competitive detection method for detection of BSA-ENRO conjugates as competitors

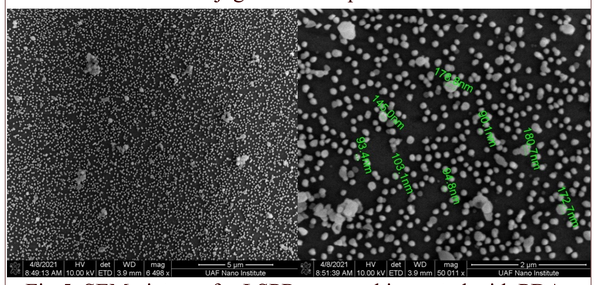


Fig 5. SEM pictures for LSPR sensor chip coated with PDA-MIP film with ENRO as template

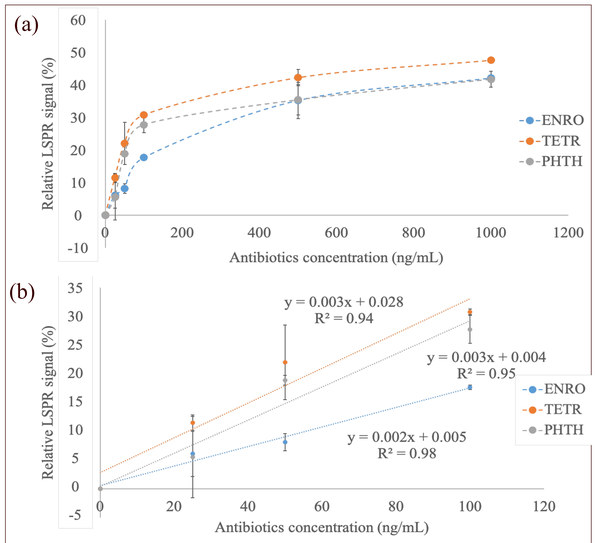


Fig 6. (a) Adsorption isotherms of ENRO, TETR and PHTH on PDA-MIP film surface obtained from injection of 0, 25, 50, 100, 500 and 1000 ng/mL standard samples; (b) Linear regression for each antibiotic target within the concentration range of 0 to 100 ng/mL

Conclusions

- The direct LSPR biosensing method with PDA-MIP film as the recognition element for detection of target antibiotics could not stimulate sensitive LSPR signals.
- The competitive method with BSA conjugates as competitors allowed a detection range of 25 to 1,000 ng/mL and a detection time of 20 min, with the LOD of 66.3, 3.7 and 71.7 ng/mL for ENRO, TETR and PHTH, respectively.
- The linear relationship between the relative LSPR signal and sample concentration for each target antibiotic within the range of 0 to 100 ng/mL was analyzed, with R^2 calculated as 0.98, 0.94 and 0.95, respectively.
- With the use of specific BSA competitors, target antibiotic was statistically discriminated from structural analogues.
- The LSPR/PDA-MIP biosensing method showed high potential for rapid and in-field detection of multi-antibiotic residues.
- Based on the analysis of standard antibiotic samples, ongoing study focuses on pretreatment and detection of spiked chicken meat, liver and kidney samples.

Acknowledgments

This research was sponsored by Walmart Foundation (0402-70013-21-0000) and supported by the Walmart Food Safety Collaboration Center.

References

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