



Portable label-free amperometric immunosensor based on decorated PVA-co-PE nanofibers for amoxicillin detection in milk



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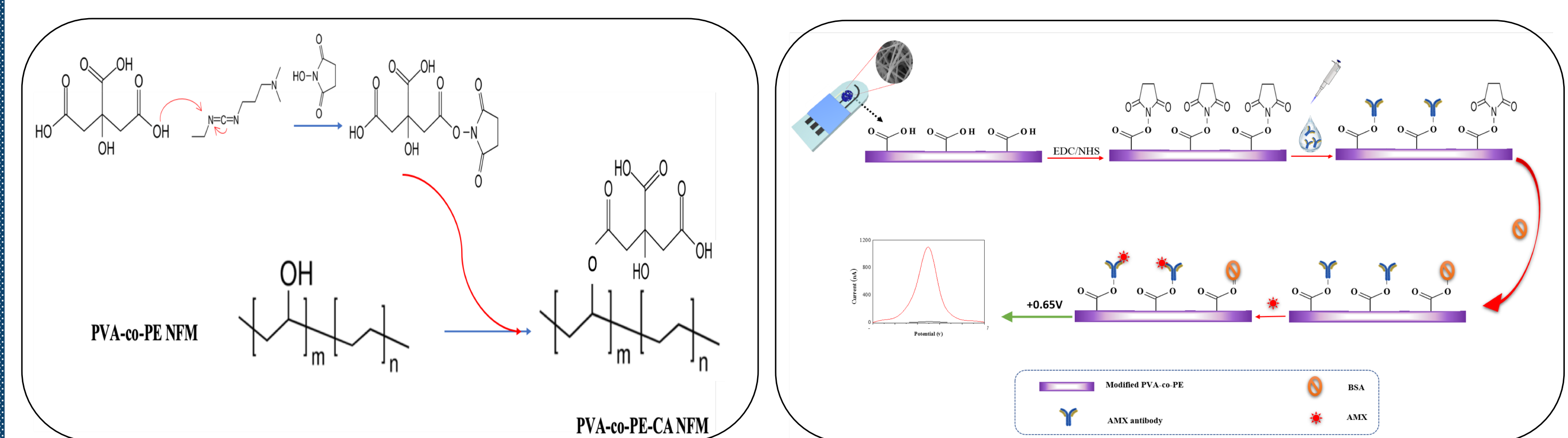
Abstract:

Milk is a highly nutritious food, and it is a source of necessary macro- and micronutrients for the growth, development and maintenance of human health. However, it may also be a source of food contaminants such as mycotoxins, pesticides and antibiotics that may cause disease. Amoxicillin (AMX) is one of the most frequently used lactam antibiotics in the world, the presence of its residues in milk poses a potential risk to public health. FDA and ECC 2377/90 have established the maximum residue limits (MRL) for AMX in milk to be 4 ng mL⁻¹. In recent years, nanofiber technology has opened new horizons for the development of the biosensor to enhance the sensitivity, selectivity, and detection time. In this work, a novel ultrasensitive label-free electrochemical immunosensor for AMX has been developed. The immunosensor was fabricated by immobilization of anti-AMX on citric acid-grafted-Poly (vinyl alcohol-co-ethylene) (PVA-co-PE) nanofibrous membrane modified screen-printed electrode. PVA-co-PE nanofibrous membrane was prepared by electrospinning technique and characterized by scanning electron microscope (SEM) and the activation step was confirmed by Fourier transform infrared spectroscopy. The employment of PVA-co-PE nanofibers comparing with PVA-co-PE casted membrane and the successful fabrication steps were investigated by electrochemical impedance spectroscopy (EIS). The amperometric response measured at 0.65 V vs. the silver pseudo-reference electrode. Under the optimal conditions, the established immunosensor exhibited high sensitivity for AMX determination in a lower range of 0.009 – 10 ng mL⁻¹ with a determination limit of 7.5 pg mL⁻¹. The proposed immunosensor was evidenced to its applicability for AMX determination in milk samples without pretreatment, showing stability, reusability and good selectivity.

1. Background

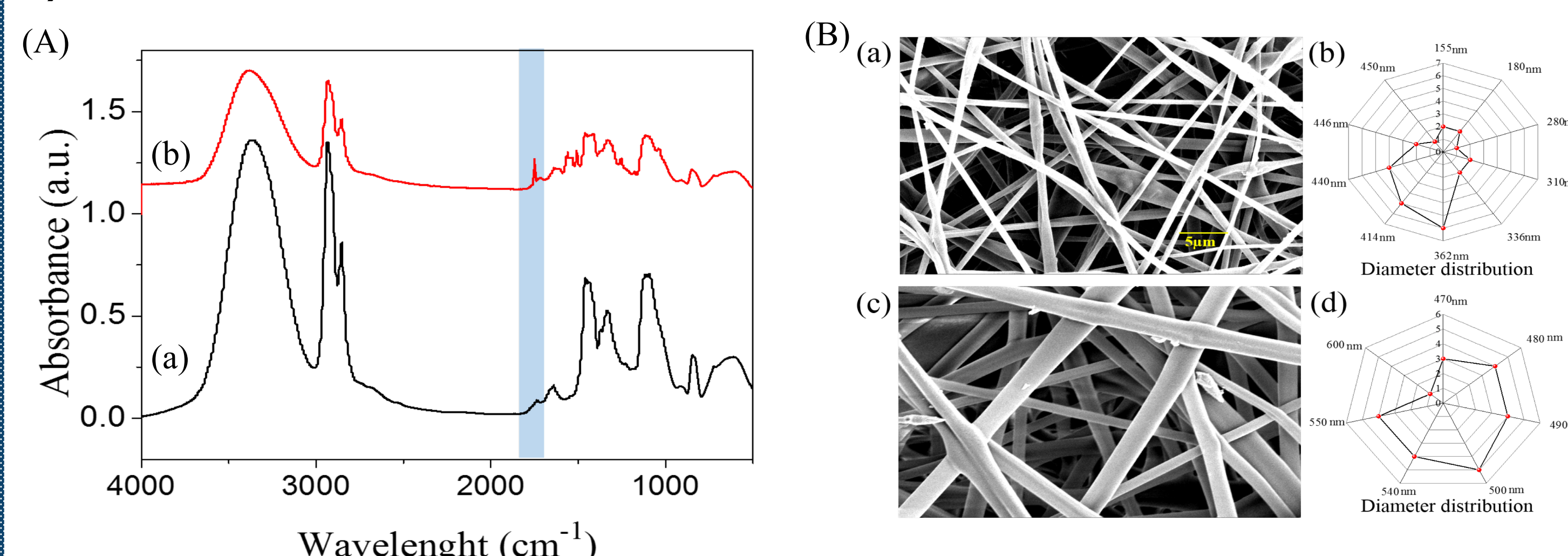
- ❖ Amoxicillin (AMX), d- α -amino-p-hydroxybenzylpenicillin tri-hydrate is one of the most frequently used lactam antibiotics in the world.
- ❖ Amoxicillin is used to treat bacterial infections in both human and animals, being one of the most used antibiotics in the treatment of bacterial infections in dairy cattle.¹
- ❖ Amoxicillin residues are found in different dairy products (i.e., milk and yogurt). The residues enter the milk supply whenever withdrawal periods are not strictly adhered to or when a cow retains the residues in its system for an extraordinary length of time.¹
- ❖ Presence of these residues in milk holds the risk of undesirable health hazards for the consumers, ranging from allergic reactions, development of mechanisms of antibiotic resistance and other related diseases.²
- ❖ Various analytical methods have been reported for the detection and separation of Amoxicillin including HPLC and capillary electrophoresis.²
- ❖ Even though, the high sensitivity and accuracy of the conventional, but on the other hand, they are not compatible for on-field sensing and detection applications and require expensive instruments, highly trained people and long time.
- ❖ Electrochemical immunosensing methods have gained growing attention as smart alternatives to conventional methods as they combine the high selectivity of the immunoassays with the high sensitivity and the low cost of electrochemical methods
- ❖ Nanofibers (NFs) are among the most promising nanomaterials, gained a growing interest during the past decade for a wide range of applications.
- ❖ NFs have opened new horizons for the biosensor development with enhanced sensitivity, selectivity, and shortened detection time due to the ultrahigh surface areas.³
- ❖ Poly(vinyl alcohol-co-ethylene) (PVA-co-PE) is a commercial polymer possessing abundant active hydroxyl groups, which can be easily activated.⁴
- ❖ To the best of our knowledge, few literature studies have reported on the construction of electrochemical immunosensor based on the electrospun polymeric nanofibers.

2. Immunosensor set-up



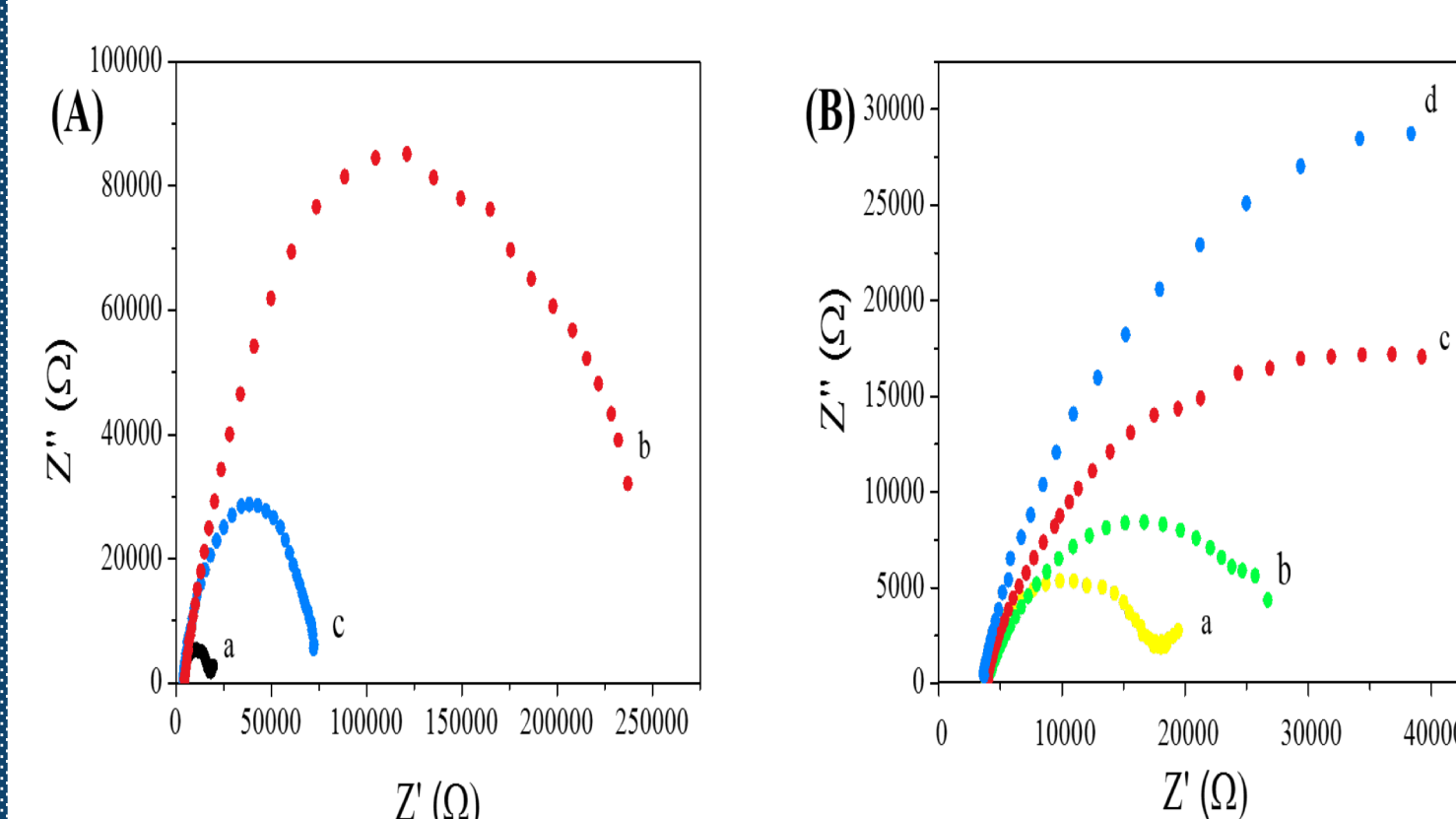
3. Results

1) Production of Functional Nanofibers Membrane:



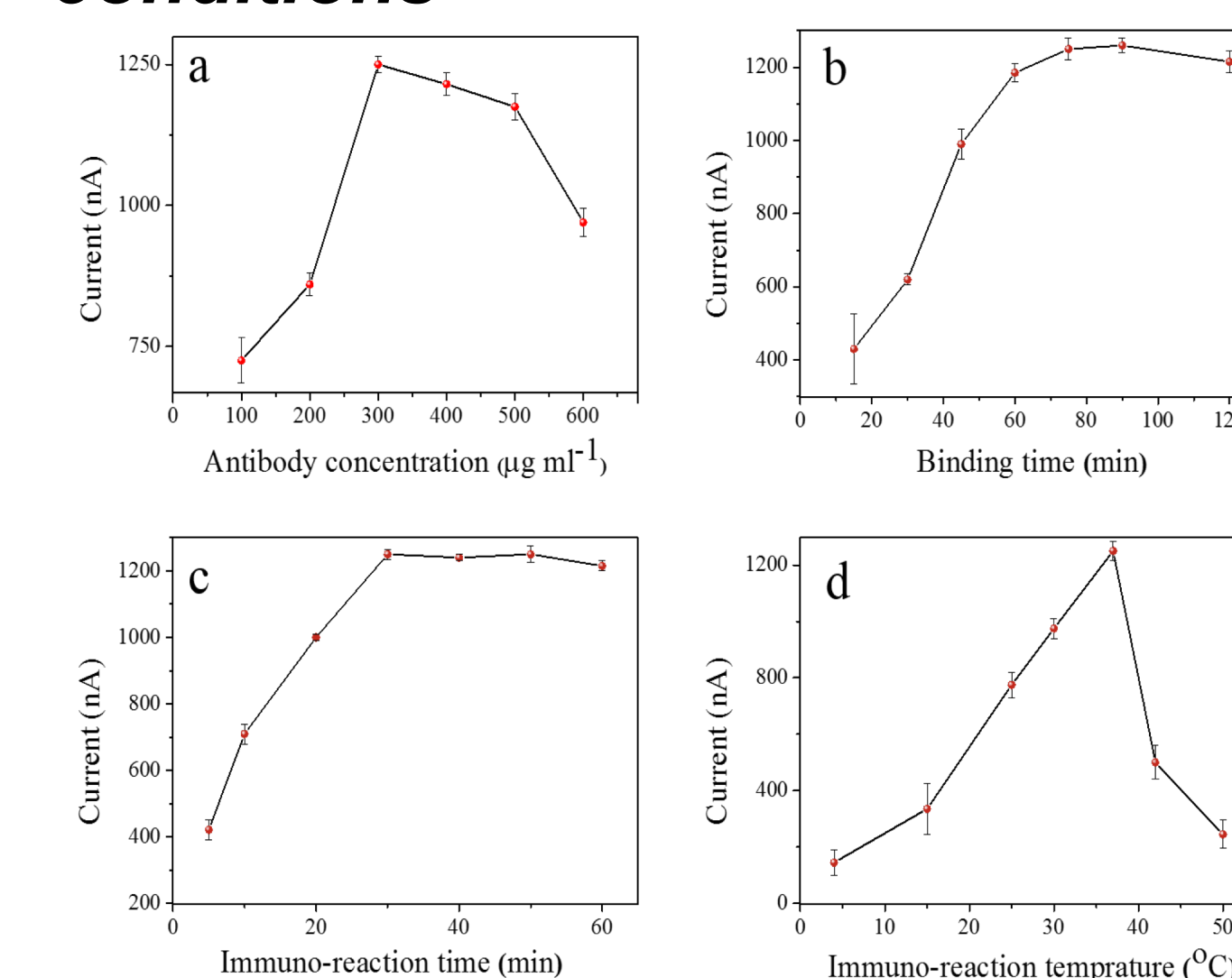
(A) FT-IR spectra of (a) pristine PVA-co-PE NFM, (b) PVA-co-PE NFM grafted with citric acid. (B) SEM images of (a) pristine PVA-co-PE NFM, and (c) PVA-co-PE-CA. Fiber diameter distributions of (b) PVA-co-PE NFM, and (d) PVA-co-PE-CA.

2) Electrochemical Characterization



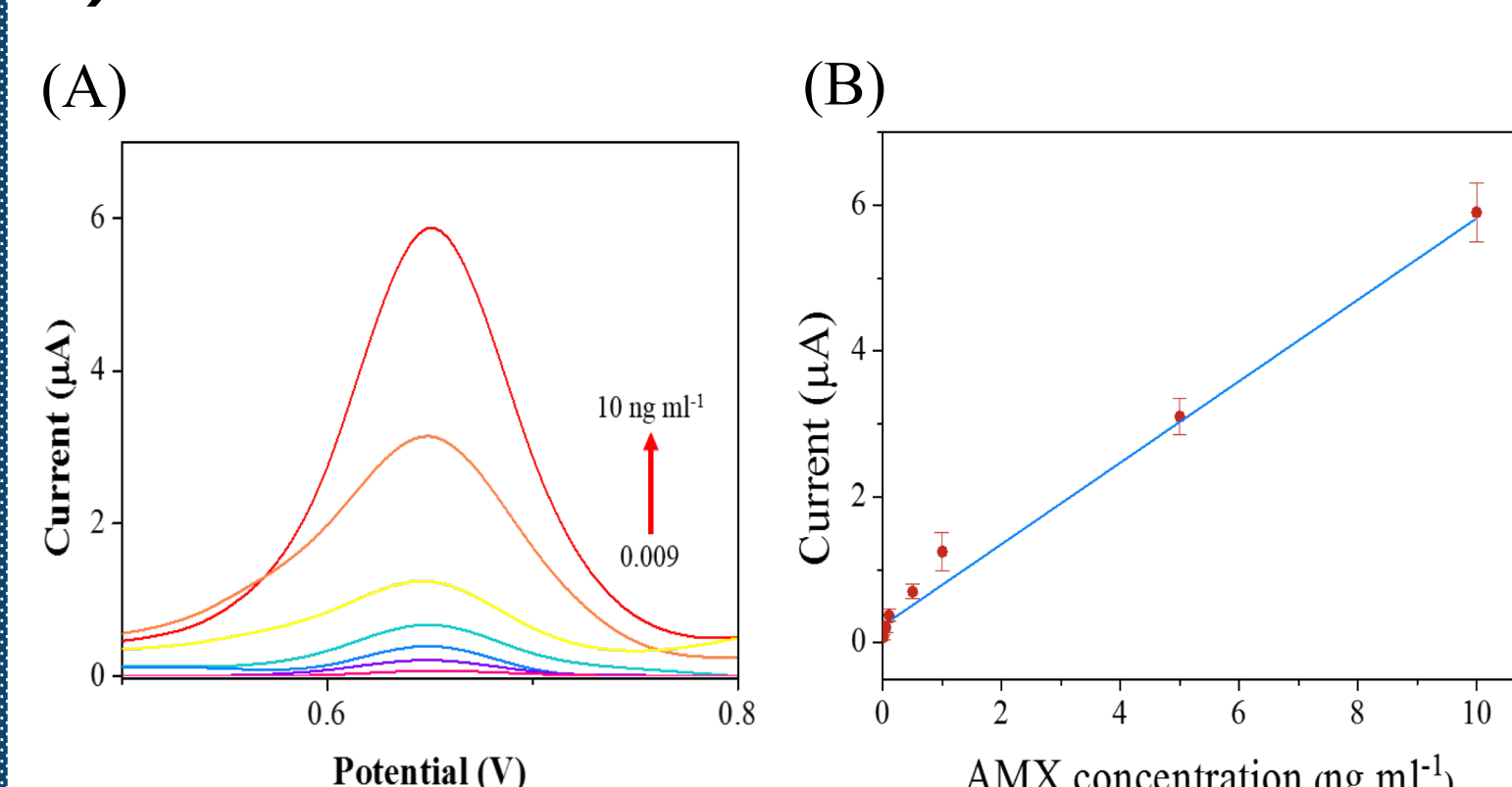
Nyquist plots of EIS in 2.5 mM [Fe(CN)₆]^{4-/3-} for: (A) SPE surface modified with (a) bare SPE, (b) PVA-co-PE CM/SPE (0.2 mm), and (c) PVA-co-PE NFM/SPE (0.2 mm). (B) SPE surface modified with (a) bare SPE, (b) PVA-co-PE NFM (0.05 mm)/SPE, (c) PVA-co-PE NFM (0.1 mm)/SPE, and (d) PVA-co-PE NFM (0.2 mm)/SPE.

3) Optimization of experimental conditions



Response to 1 ng mL⁻¹ AMX of immunosensors fabricated by using different experimental conditions: (a) antibody concentration, (b) antibody binding time, (c) immuno-reaction time and (d) immuno-reaction temperature.

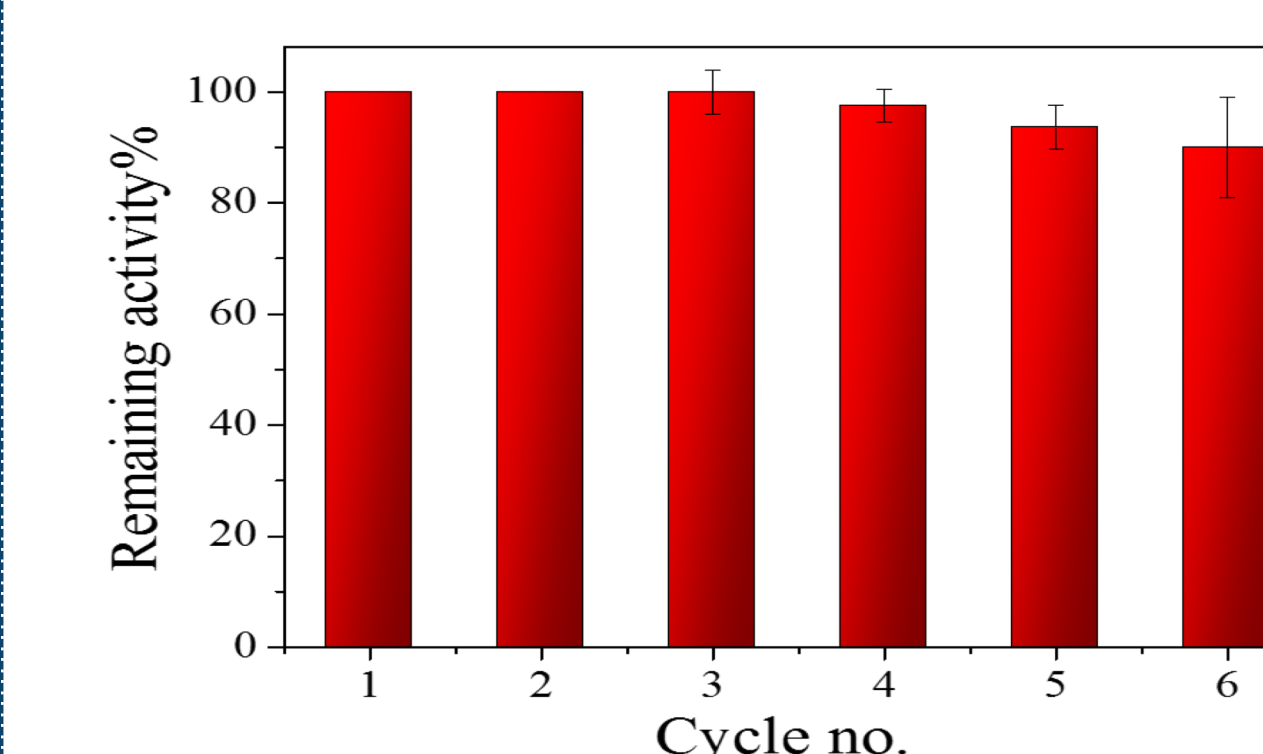
4) Detection of 3-PBA



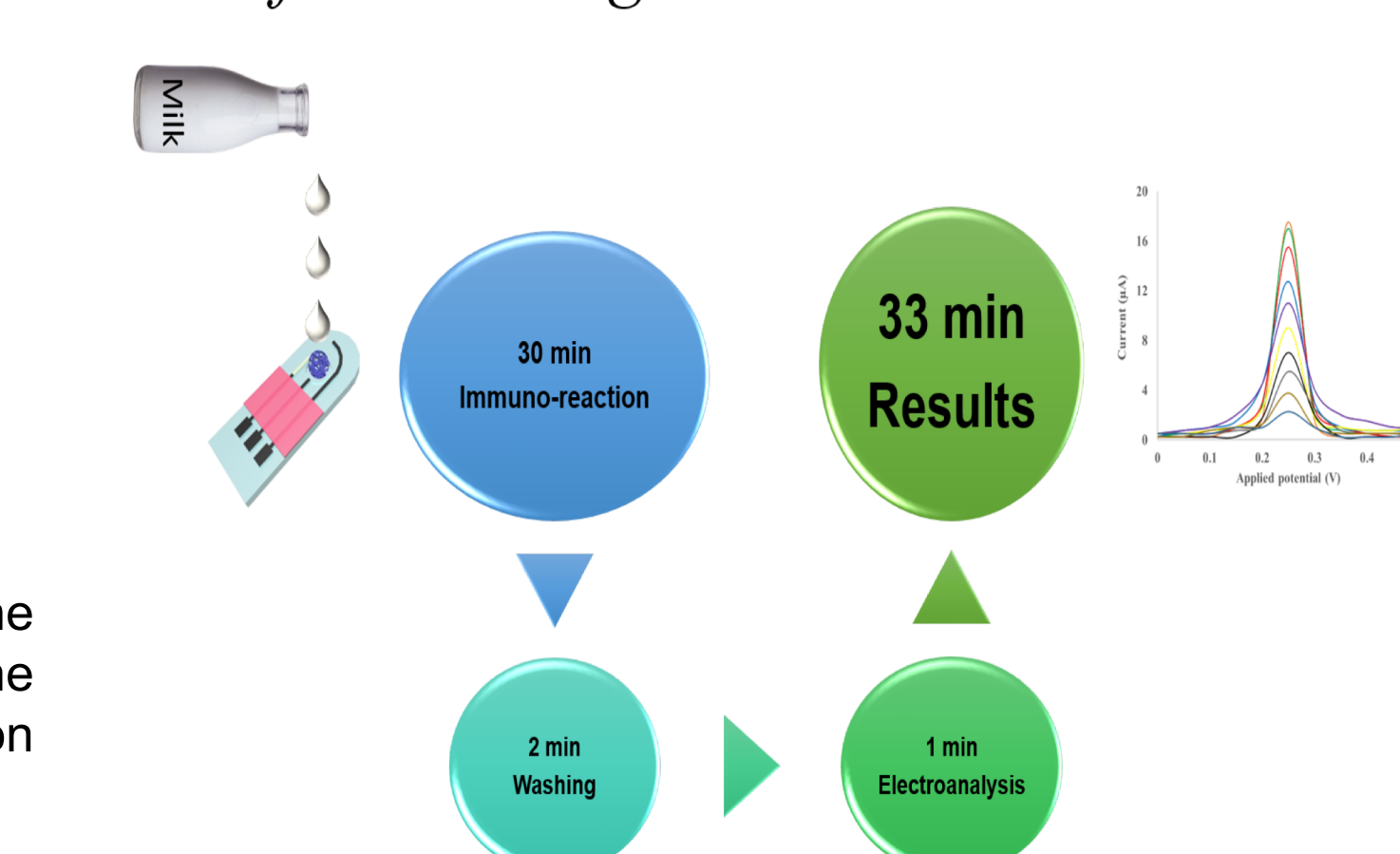
5) Selectivity & Reusability

Cross-reactivity of the developed immunosensor for different common antibiotics

Compound	Chloramphenicol	Thiamphenicol	Sulfamethazine	Gentamycin	Ciprofloxacin
C.R%	0	0	0	0	0



Time of monitoring



Reusability of the fabricated immunosensor after the regeneration by dipping the immunosensor into 0.1 M glycine hydrochloric acid buffer (pH 2.8) for 5 min after AMX detection at concentration of 0.1 ng mL⁻¹.

6) Immunosensor Applicability

Recoveries of AMX from spiked milk samples determined by the immunosensor.

Sample	Spiked concentration (ng mL ⁻¹)	Found concentration (ng mL ⁻¹)	Recovery (%)
1	0	ND	-
2	0.01	0.0092	91.7
3	0.05	0.0475	95
4	0.1	0.0961	96.1
5	1	0.93	93

4. Summary

An ultrasensitive, disposable, and rapid label-free amperometric immunosensor for AMX determination was successfully fabricated by using SPEs laminated with a layer of PVA-co-PE nanofibrous membranes. The unique structure of PVA-co-PE nanofibrous membranes improved the immunosensor response by about 4 times. The immunosensor showed very competitive analytical performances with a LOD value of AMX at 7.5 pg mL⁻¹, as well as good selectivity and stability over time. Furthermore, the feasibility of using the immunosensor in accurate determination of AMX in milk samples without any pretreatment has been demonstrated with good recovery during around 30 min.

References

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- 2 Muhammad, A., et al., 2016. Sensors, 16, 56.
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- 4 Wang, D., Sun, G., Chiou, B.S., 2007. Macromol. Mater. Eng. 292,407-414.