
بسم الله الرحمن الرحیم

A novel PEG-mediated boric acid functionalized magnetic nanomaterials based fluorescence biosensor for the detection of *Staphylococcus aureus*

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Title

- Introduction
- Methods
- Results
- Conclusion

Introduction

- *S. aureus* exists widely in nature and often causes contamination of vegetables, fruits and meat.
- Currently, the prevention of *S. aureus* infection requires accurate and rapid detection in food.
- Traditionally used conventional methods for the detection of *S. aureus* culture-based.

Fluorescence-based detection methods

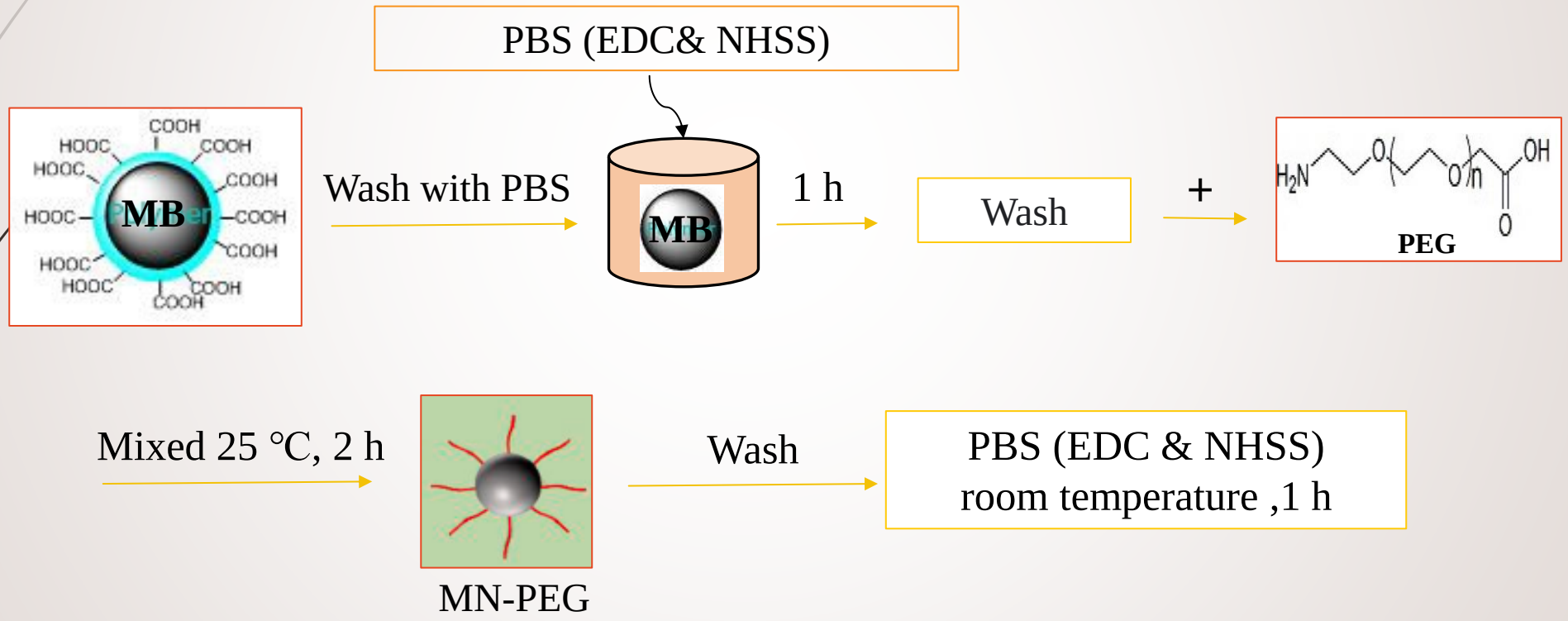
- Detection methods based on fluorescence are fast, simple and highly sensitive.
- Magnetic separation method (MB) has high specificity but low stability.
- Boric acid has good stability and is cheap, and it is used to detect bacteria.

Methods

Material

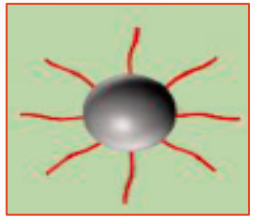
- Carboxy groups-coated magnetic beads (MBs, diameter of 180 nm)
- N- hydroxysulfosuccinimide sodium salt (NHSS)
- 1-(3- Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC)
- NH2- PEG2000-COOH
- 3-Aminophenylboronic acid
- FITC-pig IgG

Synthesis of APBA-PEG-MNs



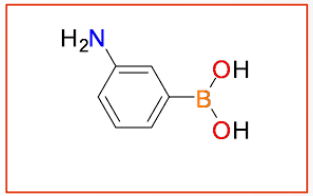
Synthesis of APBA-PEG-MNs

Wash with PBS



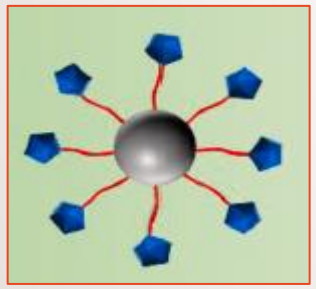
MN-PEG

+



APBA

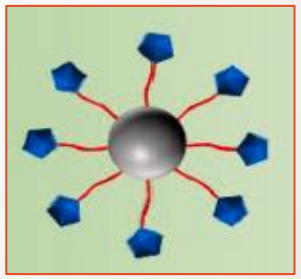
Mixed 25 °C, 2 h



APBA-PEG-MNs

Wash

remove excess APBA



APBA-PEG-MNs

store in PBS,4 ◦C

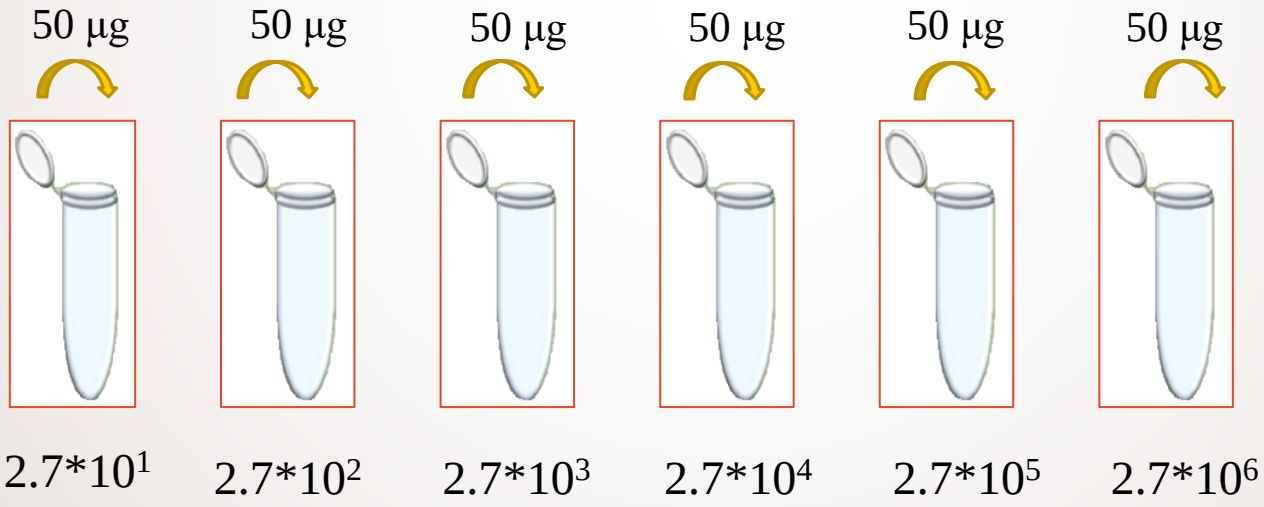
Characterization of APBA-PEG-MNs

- Electronic microscopy (SEM)
- Fourier transform infrared spectroscopy (FTIR)
- Zeta potential

Procedures of APBA-PEG-MNs separation target bacteria

1.Dilution of *S. aureus* bacteria suspension with sterile PBS.

2.50 μg of APBA-PEG-MN was added and incubated (37 °C, 20 min).



Procedures of APBA-PEG-MNs separation target bacteria

3. Under an external magnetic field, the *S. aureus*@APBA-PEG-MNs complexes were separated and resuspended with 1 mL sterile PBS.

4. 50 μL BSA solution (1 mg/mL) was added to block the nonspecific site at room temperature for 30 min.

5. *S. aureus*@APBA-PEG-MNs@BSA was washed with sterile PBS to remove excess BSA

6. Resuspend with 1 mL sterile PBS.

Procedures of APBA-PEG-MNs separation target bacteria

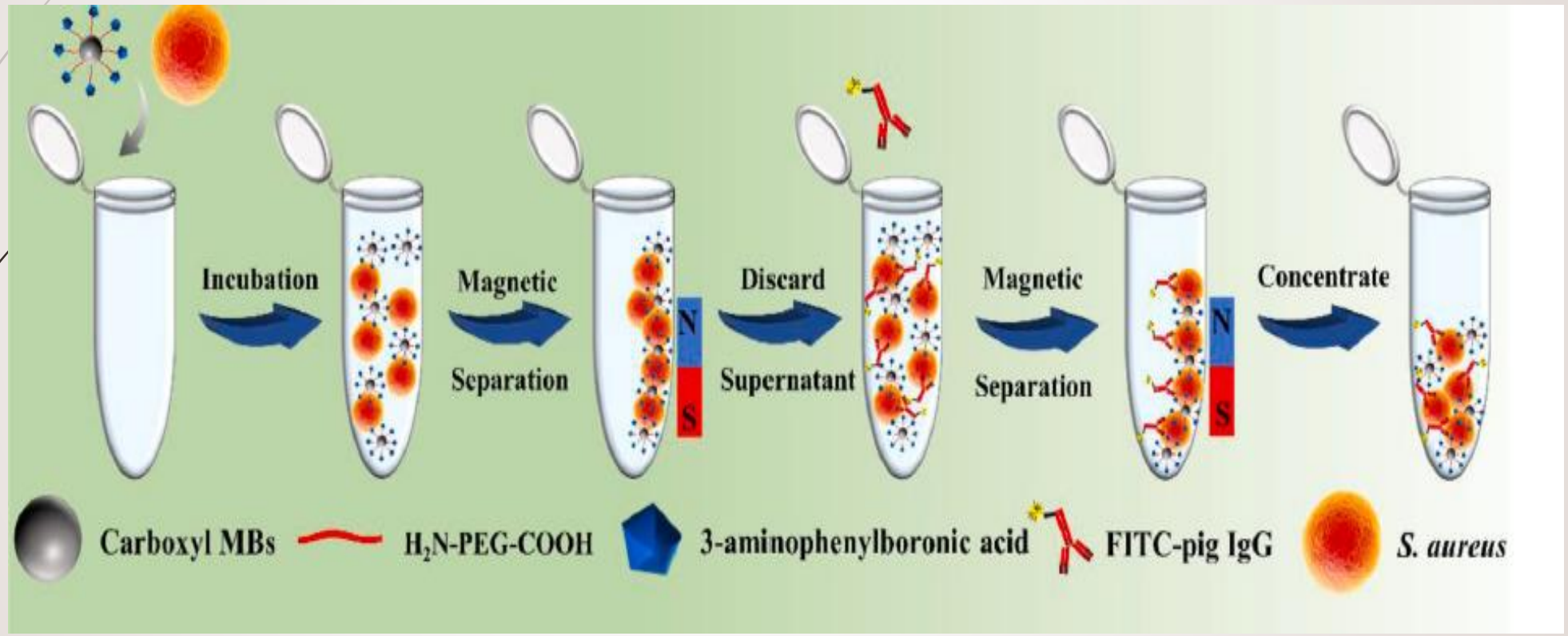
7. 2 μg FITC-pig IgG was added and incubated (37℃, 10 min).

8. The FITC-pig IgG*@S. aureus*@APBA-PEG-MNs@BSA complex was separated with a magnet and washed several times with PBS.

9. Add 200 μL of sterile PBS.

10. Measurement of fluorescence intensity of the complex solution .

Procedures of APBA-PEG-MNs separation target bacteria



Procedures of APBA-PEG-MNs separation target bacteria

To verify the separation ability :

- Dilution of supernatant and *S. aureus*@APBA-PEG-MNs complexe .
- Grown onto the LB plates at 37℃ for 12 h.
- Formula used to calculate the capture efficiency (CE) :

$$CE\ (\%) = [S1/(S1 + S2)] \times 100\%$$

S1 :number of captured colonies (CFU/mL)

S2 :number of uncaptured colonies (CFU/mL)

Detection of *S. aureus* in spiked sample

Demonstration of separation method in real samples.

- Pure *S. aureus* were added into the juice, pool water and spinach with the final concentration ranging from 10^1 to 10^6 CFU/ mL.
- Calculate CE and measure fluorescence intensity.

Specificity analysis

- *Escherichia coli, Klebsiella pneumoniae, Listeria monocytogenes, Cronobacter sakazakii, Salmonella Enteritidis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* were diluted to a concentration of 10^6 CFU/mL with sterile PBS.
- Measurement of fluorescence intensity.

Stability of APBA-PEG-MNs

1. First, prepared APBA-PEG-MNs with a concentration of 1 mg/mL were stored at 4 ℃ for 0, 7, 14, 21, and 28 days.

2. At the different time points, 50 μg APBA-PEG-MNs were added to *S. aureus* suspension with a concentration of 2.7×10^6 CFU/ mL.

3. *S. aureus*@APBA-PEG-MNs complexes were grown on the LB plate for 12 h, and the CE was calculated.

Stability of APBA-PEG-MNs

4. Sterile PBS at different pH (pH 5,6,7,8,9) was used to dilute *S. aureus* to obtain the suspension with a concentration of 2.7×10^6 CFU/mL.

5.50 μg APBA-PEG-MNs was added in *S. aureus* suspension, and the *S. aureus*@APBA-PEG-MNs complexes were cultivated at 37°C.

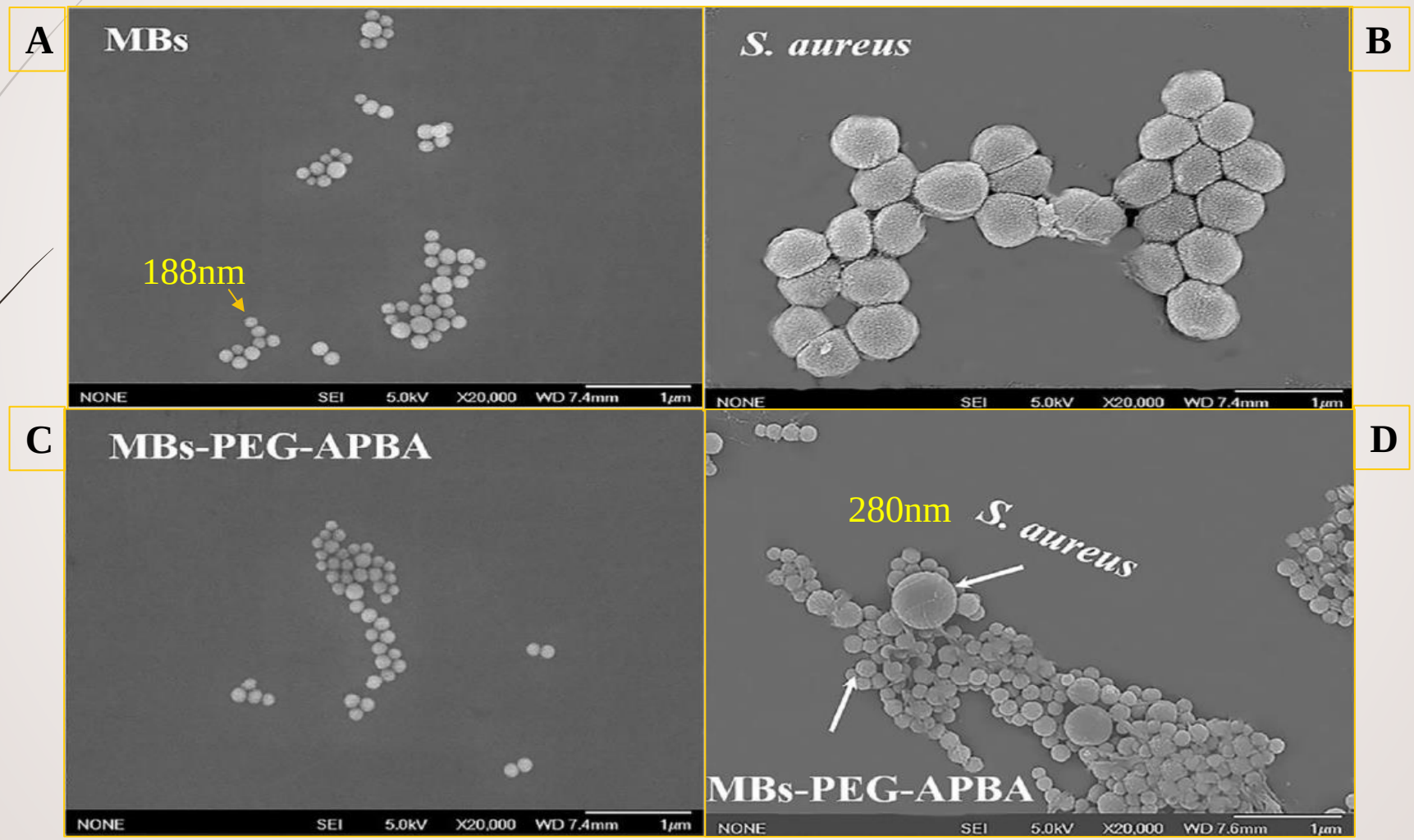
6. The CE calculate

Results

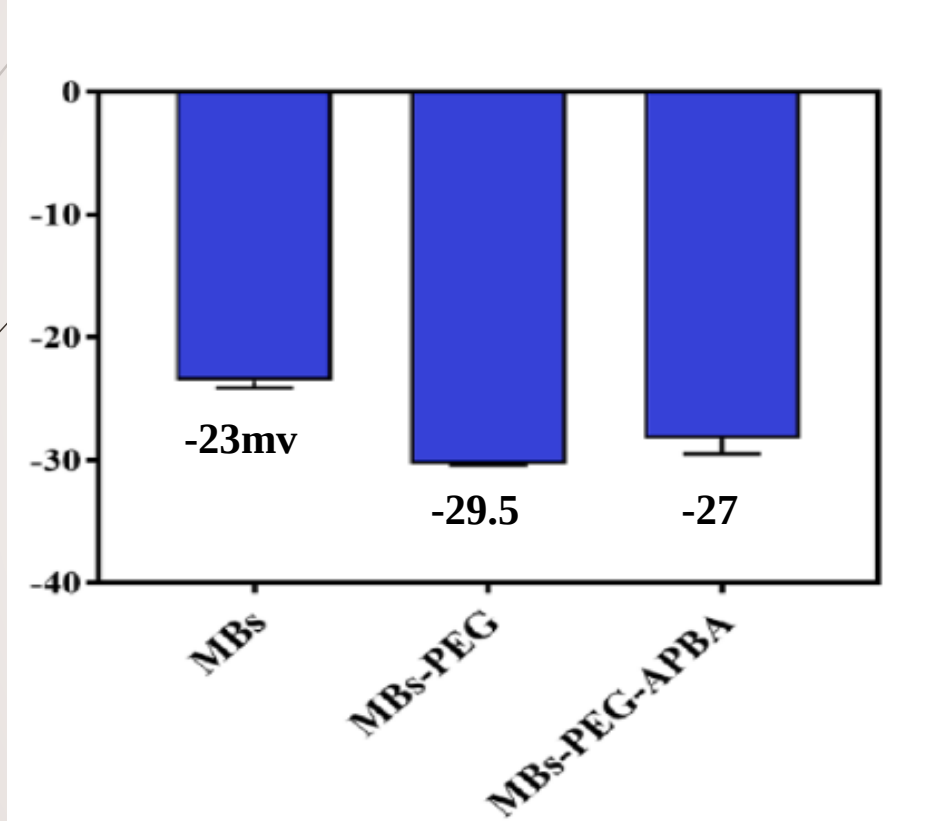
Process of the APBA-PEG-MBs based detection method for *S. aureus*

- Carboxyl groups on the surface of MBs were activated by EDC/NHSS forming a stable ester intermediate.
- Then PEG and APBA was modified on magnetic beads to form APBA-PEG-MNs.
- APBA combines with the groups in peptidoglycan.
- To improve the specificity of this method, FITC-pig IgG was added and the complex FITC-pig IgG@*S. aureus*@APBA-PEG-MNs is formed.
- Fluorescence measurement is done at 495 nm.

Characterization of APBA-PEG-MNs (SEM)

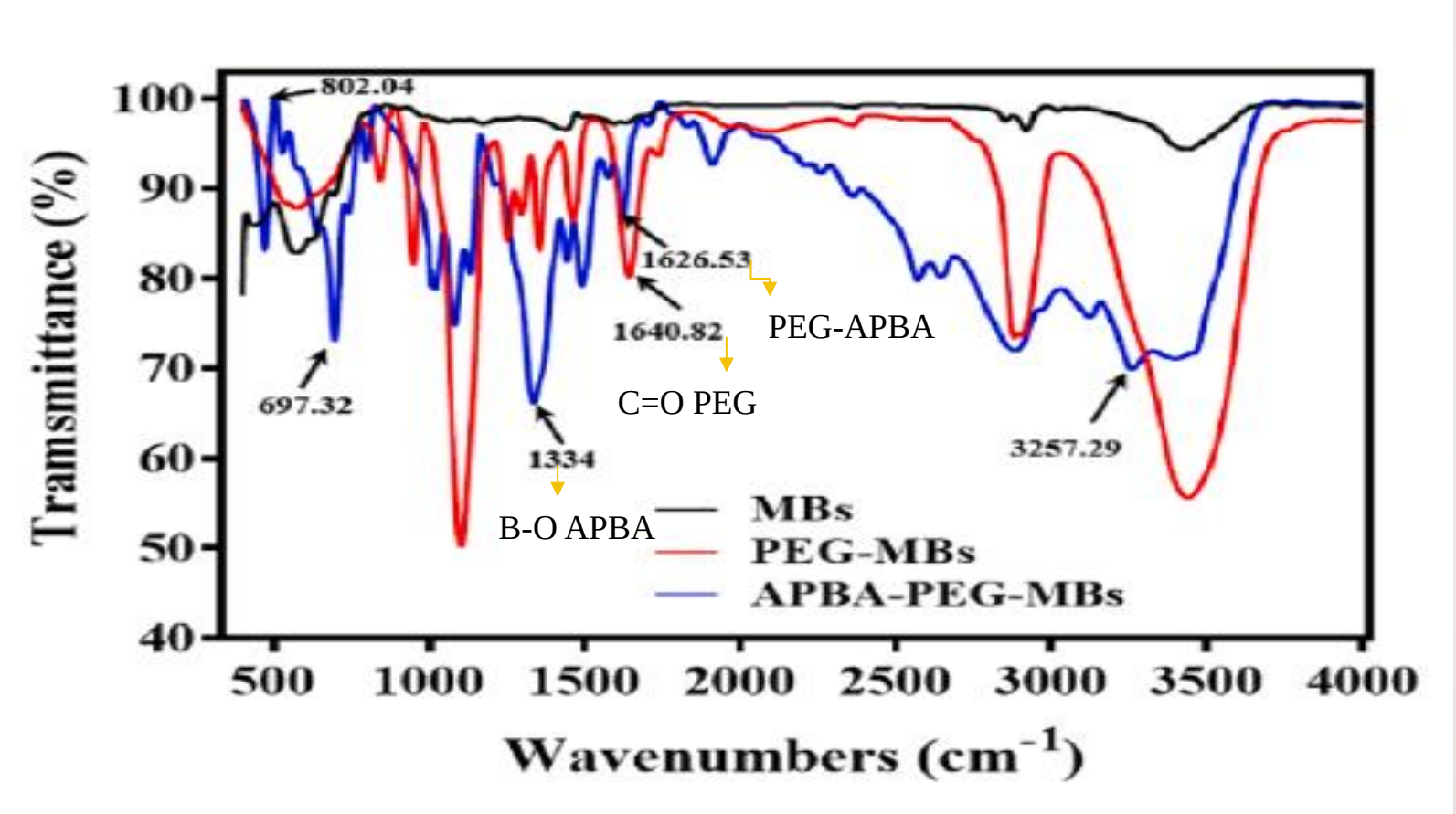


Characterization of APBA-PEG-MNs (zeta potential)



- MBs-PEG: the amino group on the PEG reacts with the carboxyl group on the MNs.
- MBs-PEG-APBA: the introduction of the boronic acid group on the APBA.

Characterization of APBA-PEG-MNs (FTIR)



Optimization conditions for *S. aureus*

- In order to have the best CE for APBA-PEG-MBs:

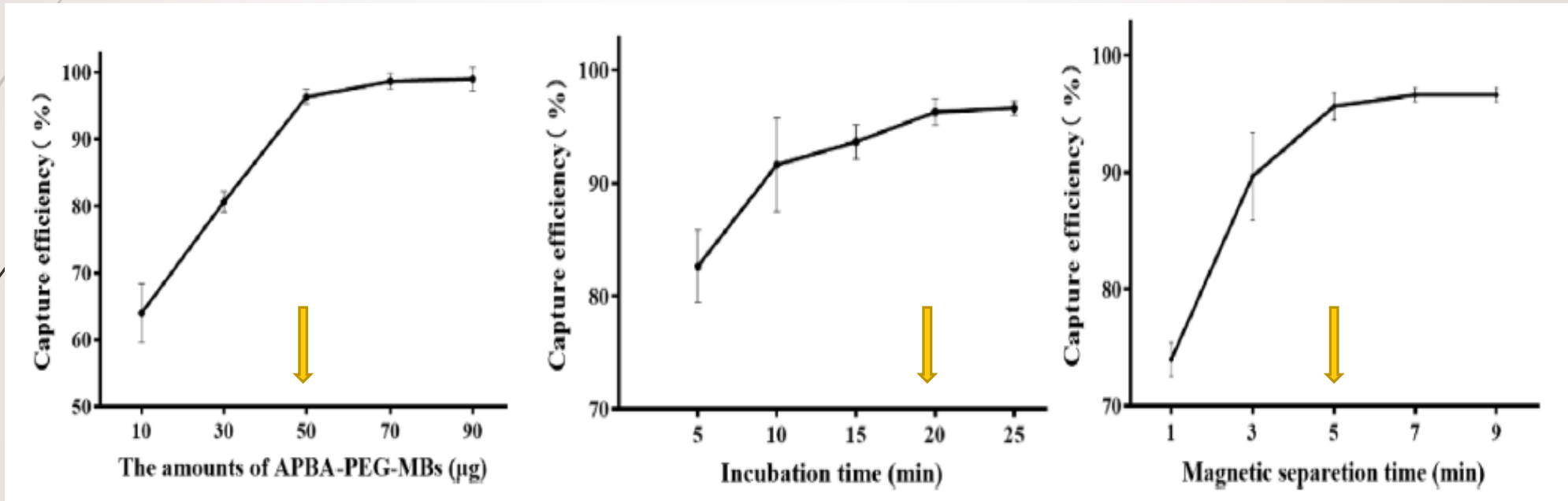
1. Optimizing the value of APBA-PEG-MN.

2. Optimizing the incubation time .

3. Optimization magnetic separation time.

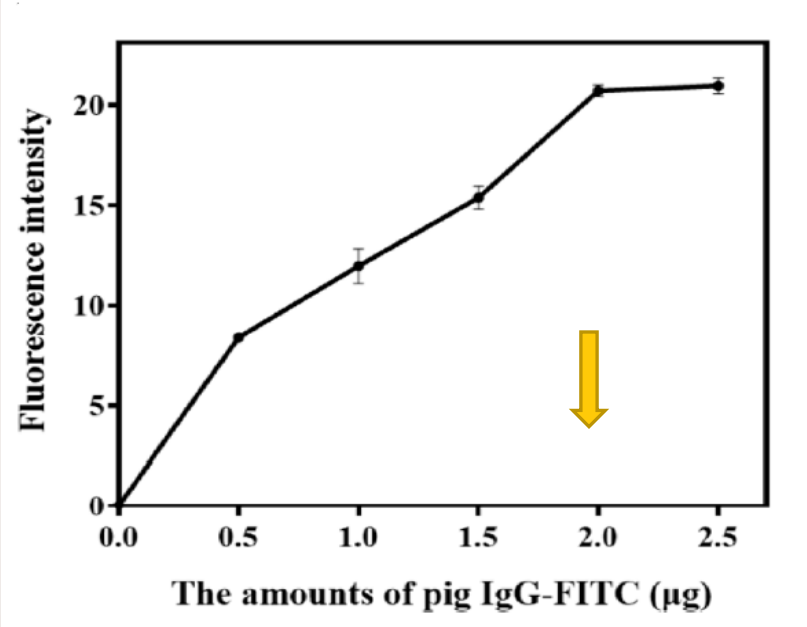
- *S. aureus* at 2.7×10^6 CFU/mL

Optimization conditions for *S. aureus*

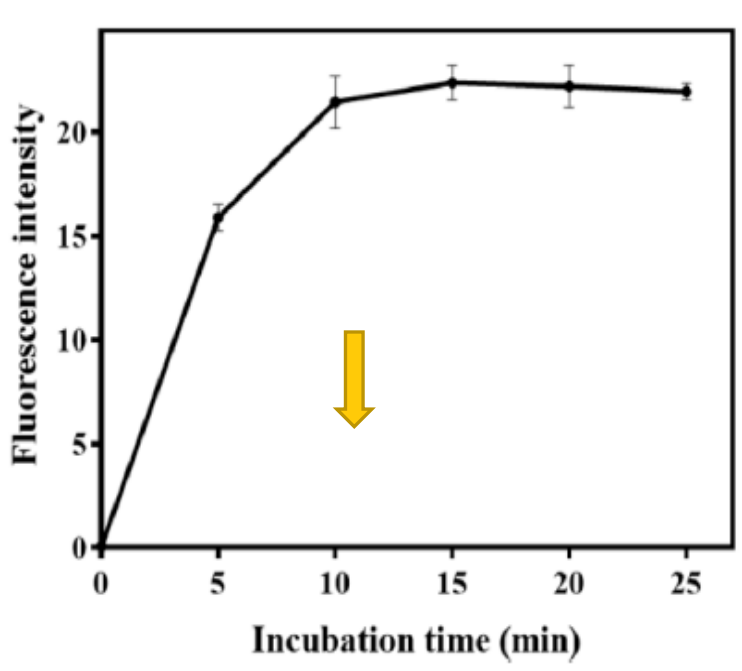


Optimization of magnetic separation experimental parameters.

Optimization of detection conditions for *S. Aureus*



The amount of FITC-pig IgG

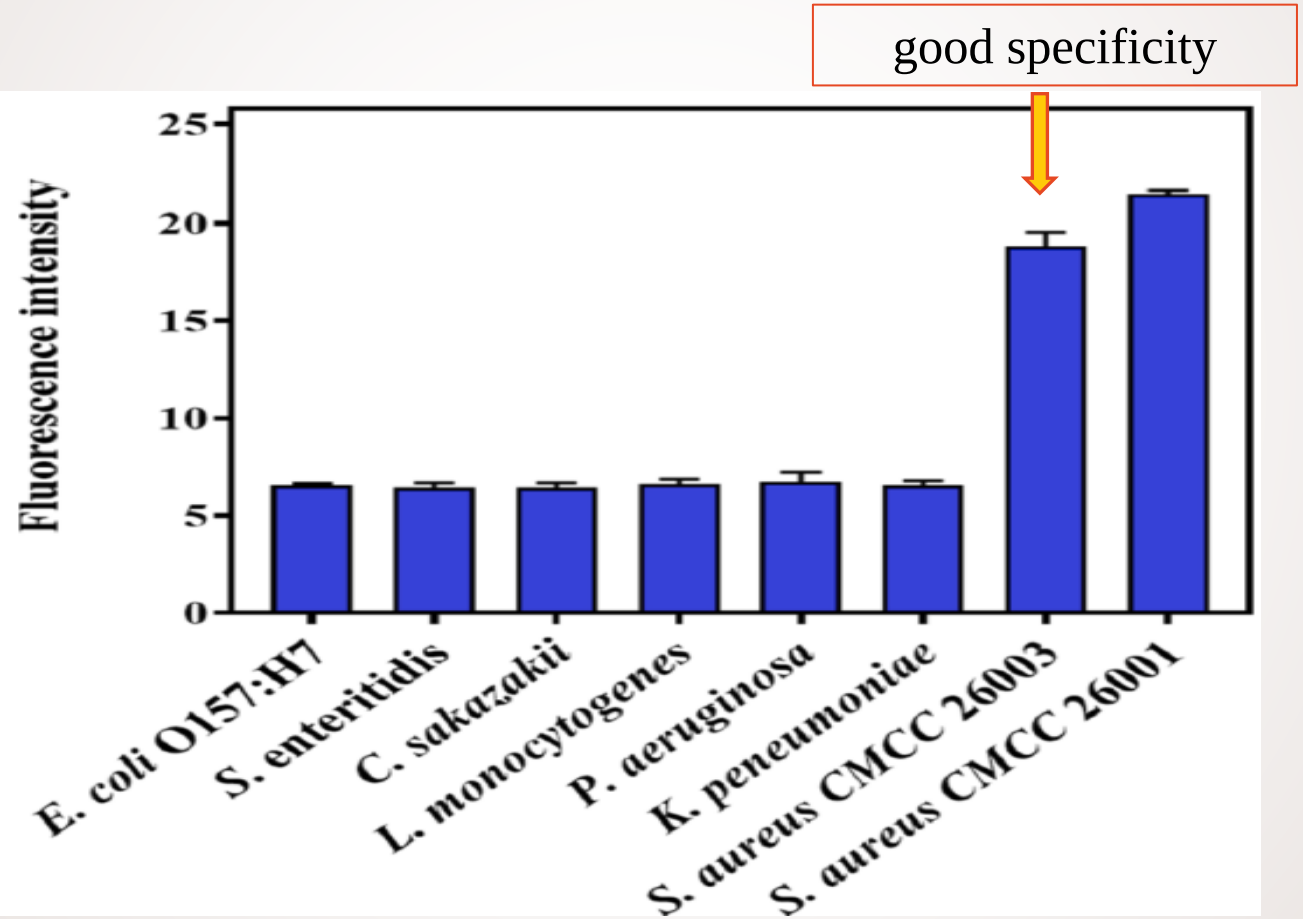


incubation time of *S. aureus*@APBA-PEG-MNs with FITC-pig IgG

Detection *s.aureus* in real samples

- The fluorescence intensity reached its highest level in the of 2.7×10^2 cfu/ml bacteria in real samples of spinach, fruit juice and pool water.
- This method has low detection limit, low cost and high stability.

Specificity of APBA-PEG-MNs



stability of APBA-PEG-MNs

Conducting separation test during storage and different pH.

- The CE had no remarkable change with storage time increases.
- The CE slightly increased or decreased when the pH changed.

Conclusion

- In this study, they were able to achieve a fast, sensitive and highly specific fluorescence detection method for *S. aureus.*
- This method allows the detection of *S. aureus* in real samples with a concentration of 2.7 × 10^2 CFU/mL in 80 minutes.

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A novel PEG-mediated boric acid functionalized magnetic nanomaterials based fluorescence biosensor for the detection of *Staphylococcus aureus*

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THANK YOU

Performance of the proposed strategy in spiked sample

Comparison of methods for detection of pathogenic bacteria by magnetic separation technology combined with existing techniques.

Recognition molecule	Target bacteria	Detection technology	LOD (CFU/mL)
Teicoplanin	*Staphylococcus aureus*	chemiluminescent	1.5×10^2
Antibody	*Escherichia coli O157:H7*	immunochromatographic	3×10^3
Vancomycin	Methicillin-resistant *Staphylococcus aureus*	mPCR	10^3
Aptamer	*Alicyclobacillus acidoterrestris*	Fluorescence	10^3
APBA	*Staphylococcus aureus*	Fluorescence	2.7×10^2