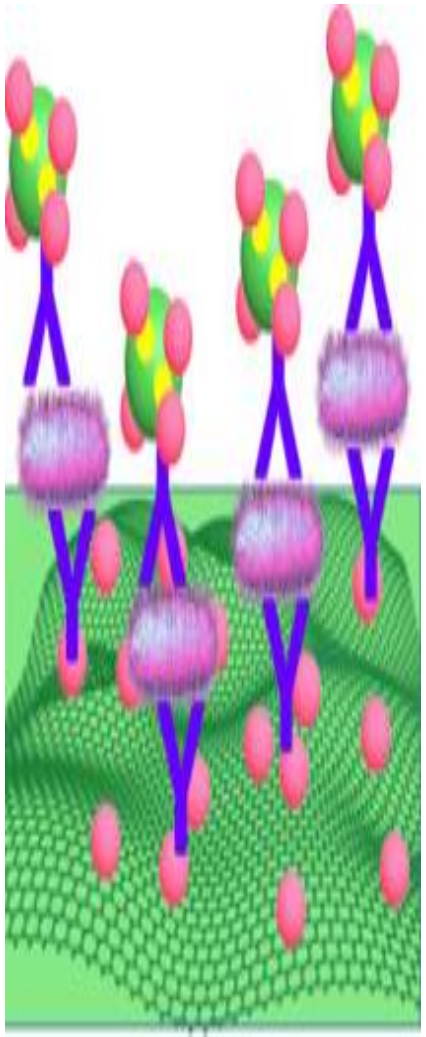

In the Name of God



Gold nanoparticles-based multifunctional nanoconjugates for highly sensitive and enzyme-free detection of E.coli K12

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Contents

Introduction

- Accurate and robust detection of pathogenic bacteria in food and water remains an important issue
- That is because some of the strains of E.coli
- According the estimation of World Health Organization (WHO), 1.5 million children lose their lives owing to the diarrheal diseases every year
- In the past decades, the methods for the detection of E.coli have been extensively explored
- Though reliable results can be obtained from these methods

Introduction

- nanocomposite has been prepared by assembling well-distributed gold nanoparticles (AuNPs) ,polypyrrole-reduced graphene oxide (PPy-rGO)

- This serves as a platform for immobilization of a capture antibody

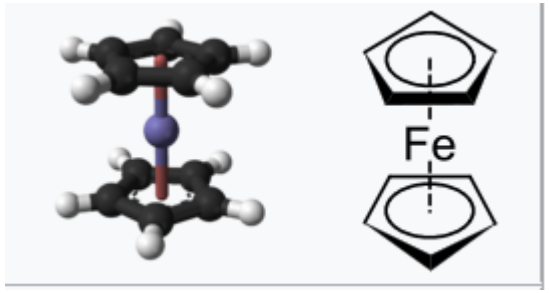
- This nanocomposite has
  - high surface area
  - good conductivity
  - biocompatibility

Material and methods

1. *Chemicals and Materials*
2. Preparation of PPy-rGO/AuNPs nanocomposite and PPy@Fc/AuNPs antibody label
3. *Preparation of the E.coli immunosensor*
4. *Apparatus*

1- Chemicals and Materials

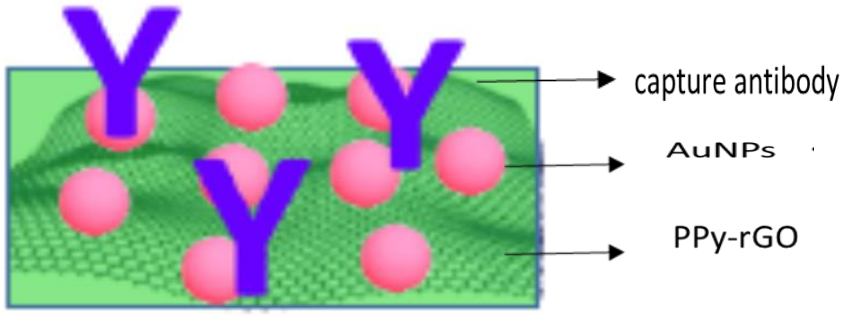
1. sodium citrate
2. ammonium peroxydisulfate (APS) and potassium ferricyanide
3. Lipoic acid n-hydroxysuccinimide ester (LPA)
4. Graphene oxide (GO) was prepared using Hummers' method
5. polypyrrole (PPy)
6. assembling well-distributed gold nanoparticles (AuNPs)
7. Polyclonal antibodies of E. coli
8. E.coli K12
9. Ferrocene (Fc)-labeling



2- Preparation of PPy-rGO/AuNPs nanocomposite and PPy@Fc/AuNPs antibody label

Preparation of PPy-rGO/AuNPs nanocomposite

- Graphene oxide → dw → ultrasonicated
- pyrrole and HCl → stirred
- APS → stirred
- AuNPs → centrifuged → with methanol → 60 ºC for 12 h



Preparation of PPy@Fc/AuNPs antibody label

- Pyrrole + HCl + SDS → dw → stirred
- Fc → ethanol → added to solution → stirred
- APS + AuNPs → added to solution → centrifuged → rinsed with water → at 60 ºC for 12 h.
- PPy@Fc/AuNPs + APS → 4h at 4 ºC → centrifuged → rinsed with water
- PPy@Fc/AuNPs + Ab2 → for 12 h → rinsed with PBS
- Finally, the PPy@Fc/AuNPs-Ab2 label was stored in refrigerator at 4 ºC for use

1- Chemicals and Material

1- Ferrocene (Fc)-labeling

- the Fc redox label is encapsulated within the matrix of the Ppy nanoparticles and used as an enzyme-free label to generate a current response
- Ferrocene (Fc)-labeling of the detection antibody is an effective way to generate a quantifiable current signal in sandwich assays

2- Polypyrrole:

- Ppy is an ideal substrate for nanoparticle modification achieve a disperse AuNPs distribution
- Ppy has good electrical conductivity, environmental stability, and biocompatibility
- Polymerization of pyrrole on graphene oxide in the presence of ammonium peroxydisulfate (APS) generates a positively charged surface

3- AuNPs

- prepared using citrate as reducing agent, will carry a negative surface charge and decorate the surface of the positively charged PPy-graphene composite.

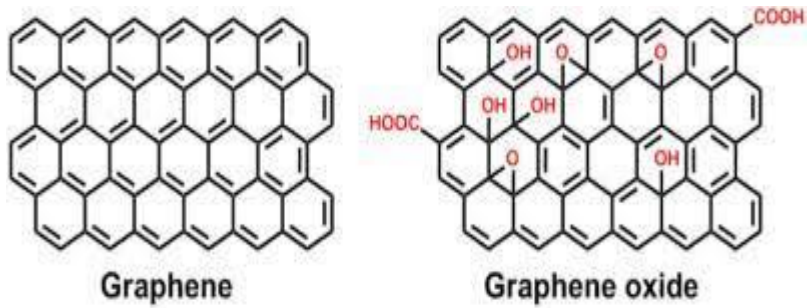
1- Chemicals and Material

4- Graphene oxide:

- Graphite oxide is a compound of carbon, oxygen, and hydrogen in variable ratios
- can be dispersed by sonication to yield monomolecular sheets, known as graphene oxide by analogy to graphene, the single-layer form of graphite

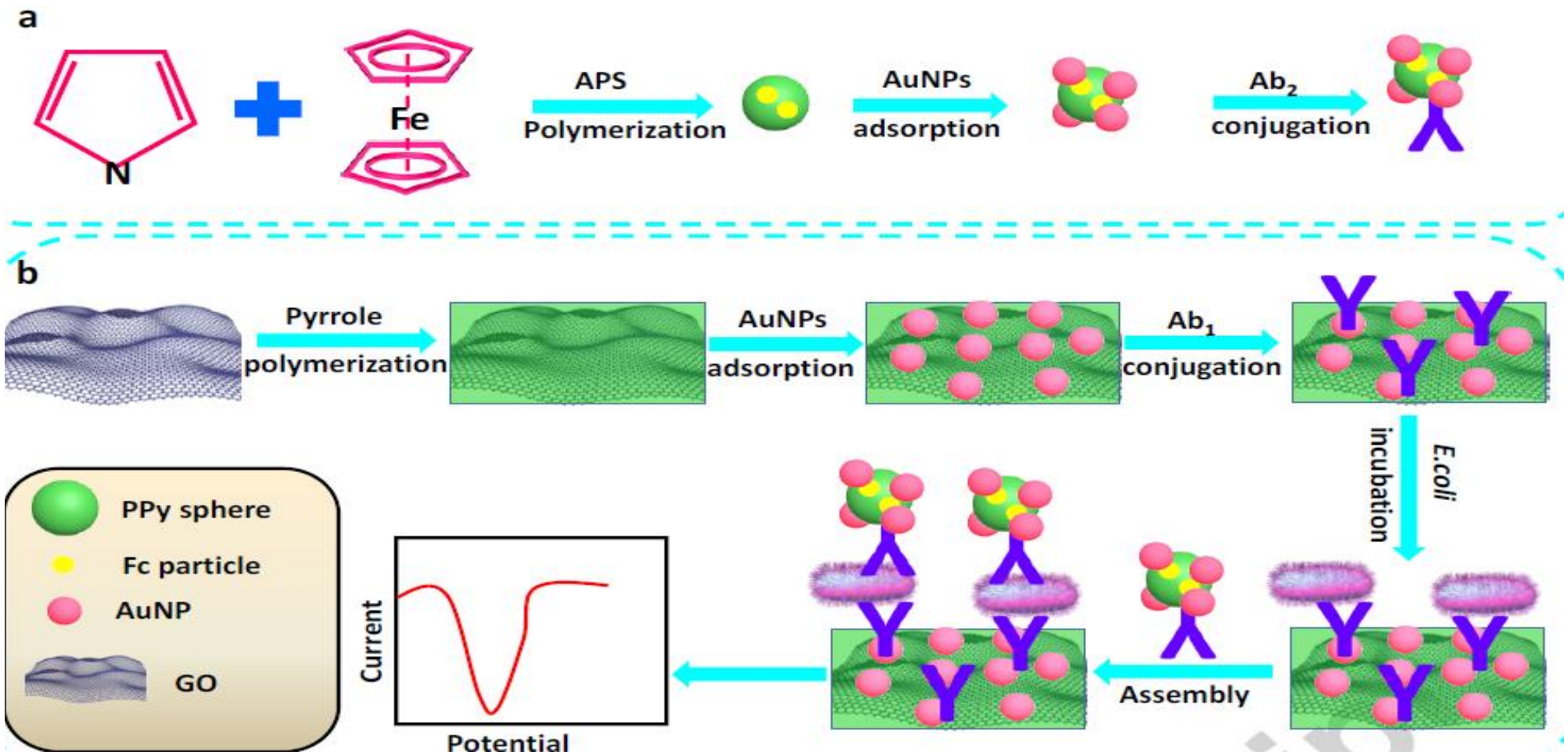
5- Polyclonal antibodies

- The attachment of a peroxidase-labeled detection antibody (Ab2).
- Ferrocene (Fc)-labeling of the detection antibody is an effective way to generate a quantifiable current signal in sandwich assays



Graphene Graphene oxide

1- Chemicals and Material



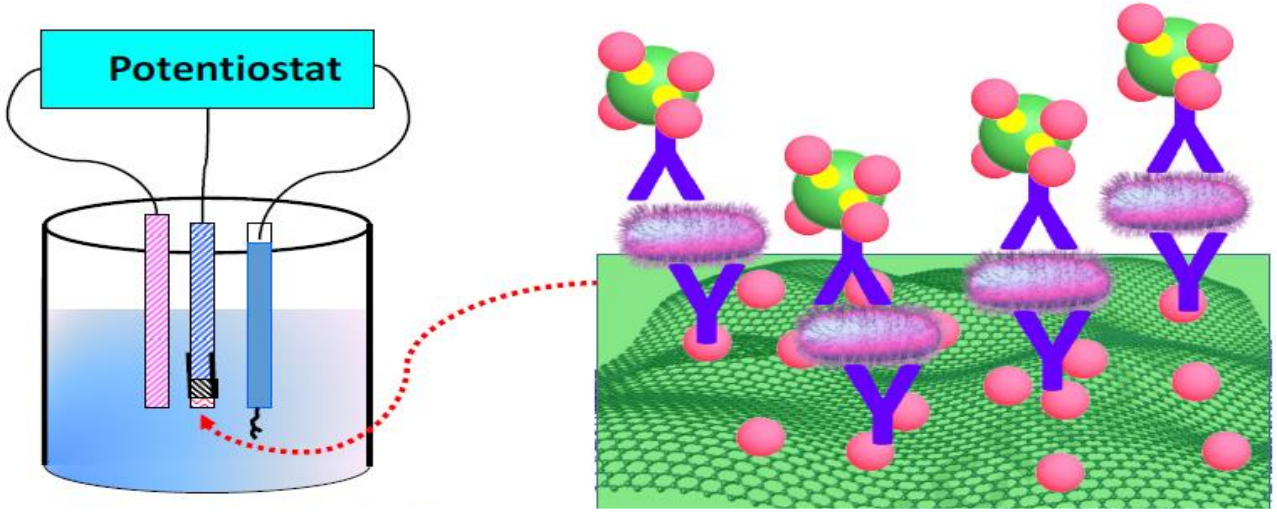
3. Preparation of the E.coli immunosensor

1. PPy-rGO/AuNPs nanocomposite (DMF) ultrasonication for 1 h.
2. Then the modified electrode was incubated in 0.01 M LPA solution for 4 h
3. Then the electrode was incubated in Ab1 solution
4. Finally, the electrode was rinsed again with PBS and kept at 4 ºC for use.

3. Preparation of the E.coli immunosensor

❑ **To detect E.coli K12**

- the PPy-rGO/AuNPs nanocomposite modified electrode was incubated a series of E. coli K12
- Then the electrode was rinsed with PBS to remove the physically attached cells.
- Finally the electrode was incubated in PPy@Fc/AuNPs-Ab2 suspension for 2h at 4c



4- Apparatus

- The Fourier transform infrared (FTIR) spectra
- The Raman spectra
- transmission electron microscope(TEM)
- electrochemical station

Results and discussion

1. *Characterization of the PPy-rGO/AuNPs nanocomposite*
   - *The Fourier transform infrared (FTIR) spectr*
   - *The Raman spectra*
   - *transmission electron microscope(TEM)*
2. *Performance of the immunoassay to E.coli* K12
3. Real sample testing
4. Selectivity, reproducibility and stability

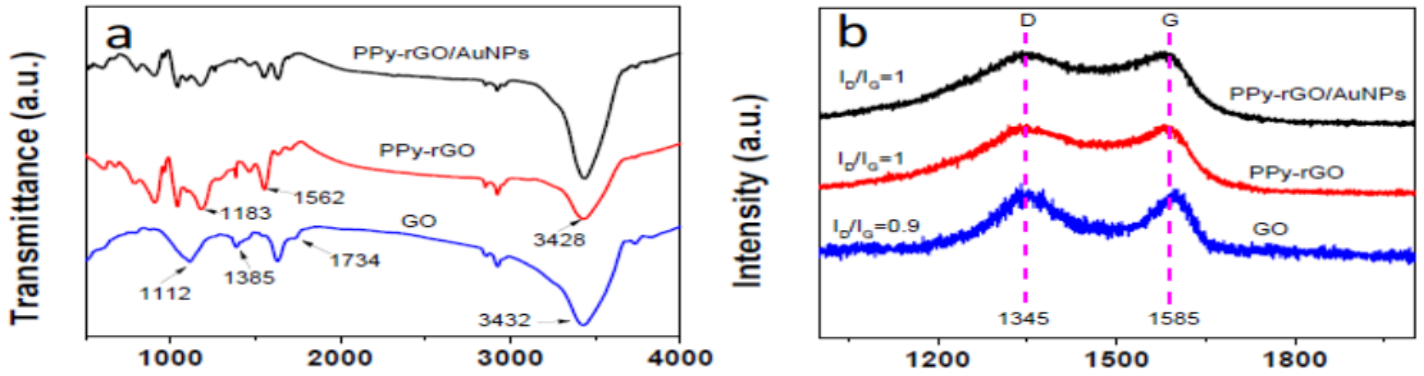
1. Characterization of the PPy-rGO/AuNPs nanocomposite

a. The nanocomposite was investigated by FTIR shown

- These peaks become weaker and disappeared in the spectrum of PPy-rGO and PPy-rGO/AuNPs, which is due to reduction of the GO by pyrrole
- indicating that PPy was successfully deposited on the graphene surface

b. The samples were further studied by Raman spectroscopy

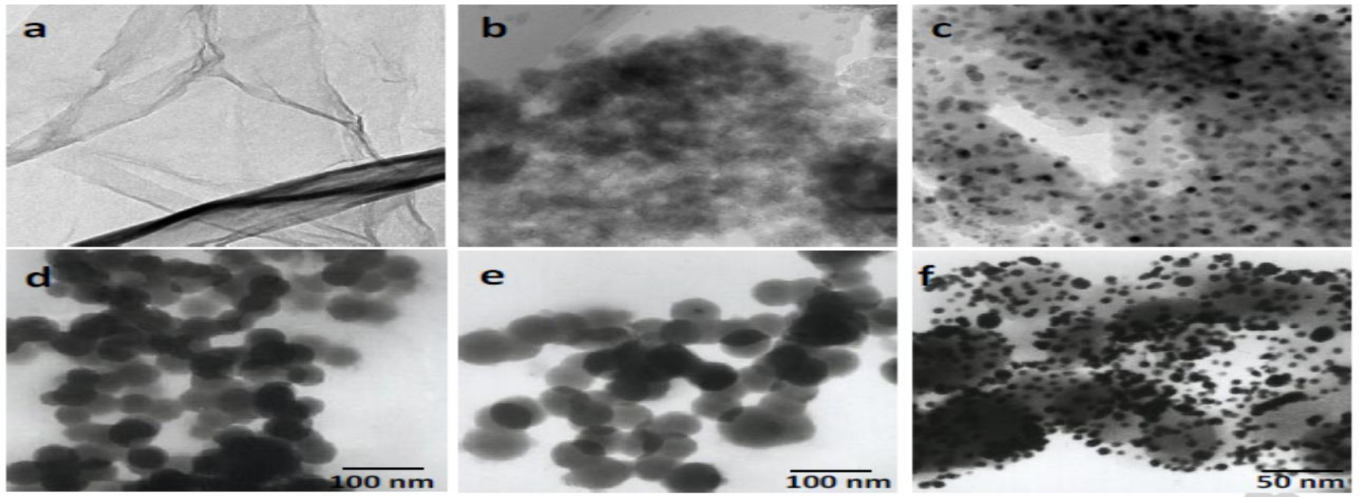
- a graphite-like band (G-band) and a disorder-induced band (D-band) were observed in all the samples, suggesting the coexistence of disordered and graphitic carbons



1. Characterization of the PPy-rGO/AuNPs nanocomposite

d- transmission electron microscope

- GO exhibits a wrinkled surface, which is the typical characterization of.a
- After the deposition of PPy on the surface of GO, the surface becomes rough .b
- The AuNPs was uniformly distributed on the surface of PPy-rGO without obvious aggregation.c
- For comparasion, the AuNPs was attached on the surface of GO without the assitance of Ppy
- However, it is caused severe aggregation on a solid surface due to high surface energy



2. Performance of the immunoassay to E.coli K12

- Fc is always used as the electrochemical probe
- When the bacteria are bound to a modified surface

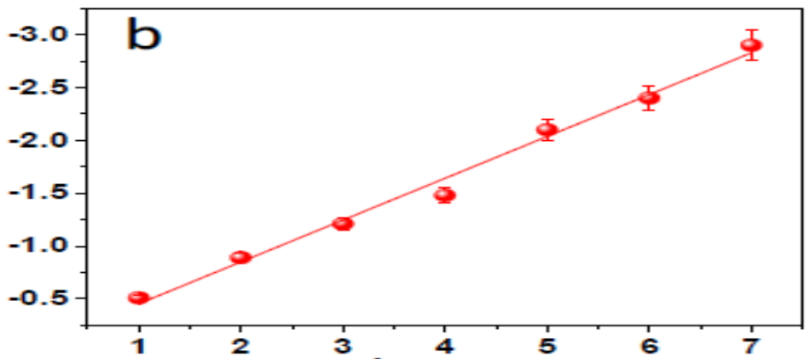
❑ a series of control experiments on the immunosensor were carried out.

1. The effect of the PPy-rGO/AuNPs loading on the current response of the immunosensor
2. with increasing the Ab1 concentration, the current response increased
3. The effect of E.coli K12 incubation time
4. The ionic strength of the PBS buffer is also investigated with the aim of improving the sensitivity of the immunosensor

2. Performance of the immunoassay to E.coli K12

5- the current response of the immunosensor to varied concentrations of E.coli K12 in PBS solution was investigated

- The peak current of the curve increased with the increasing of the E.coli K12 concentration
- The detection limit calculated is 10 CFU



Immunosensor platform	Bio-receptor of immobilization	Detection limit (CFU mL^{-1})	Linear range (CFU mL^{-1})	Ref.
Electrochemical	Antibody	1.0×10^{2}	1.0×10^{2}–1.0×10^{6}	[12]
PCR	DNA	1.0×10^{3}	1.0×10^{1}–1.0×10^{7}	[51]
Electrochemical	Antibody	1.0×10^{2}	1.0×10^{2}–1.0×10^{5}	[52]
Fluorescence	Bacteriophage	-	1.0×10^{4}–1.0×10^{5}	[53]
Spectroscopy	Antibody	1.0×10^{1}	1.0×10^{1}–1.0×10^{7}	[54]
Optical	Antibody	1.0×10^{3}	1.0×10^{3}–1.0×10^{5}	[55]
Gravimetric	Antibody	1.0×10^{3}	1.0×10^{5}–6.2×10^{7}	[56]
Electrochemical	Antibody	1.0×10^{1}	1.0×10^{1}-1.0×10^{7}	This study

3. Real sample testing

- The performance of the immunosensors in the real-to-life conditions was investigated by standard addition method.
- Water and milk samples were spiked with E.coli K12.
- 0.1 M KCl was added to the solution and used as the supporting electrolyte.
- These results demonstrate the immunosensors with these nanocomposites are suitable for real sample analysis

Samples	Number	Added (CFU mL^{-1})	Detected (CFU mL^{-1})	Average recovery
Tap water (City of Toronto)	1	$(1.5\pm0.1)\times10^4$	$(1.5\pm0.1)\times10^4$	100%
	2	$(2.5\pm0.2)\times10^4$	$(2.6\pm0.1)\times10^4$	104%
	3	$(4.0\pm0.3)\times10^4$	$(4.1\pm0.2)\times10^4$	102%
	4	$(5.0\pm0.1)\times10^4$	$(4.8\pm0.1)\times10^4$	96%
	5	$(8.0\pm0.1)\times10^4$	$(8.1\pm0.2)\times10^4$	101%
Milk (Grocery Store, Toronto)	1	$(2.0\pm0.1)\times10^4$	$(2.1\pm0.1)\times10^4$	105%
	2	$(3.5\pm0.2)\times10^4$	$(3.4\pm0.2)\times10^4$	97%
	3	$(5.0\pm0.1)\times10^4$	$(4.7\pm0.1)\times10^4$	94%
	4	$(6.0\pm0.3)\times10^4$	$(5.9\pm0.1)\times10^4$	98%
	5	$(7.5\pm0.3)\times10^4$	$(7.2\pm0.2)\times10^4$	96%

4. Selectivity, reproducibility and stability

a. selectivity

- To assess the selectivity of the proposed immunosensor
- the current signal for E. faecalis and B. subtilis which is significantly lower than that of E.coli K12
- These results demonstrate that the immunosensor has good selectivity and can perform well in a mixed cell culture.
- The most common electrochemical interfering Species

b. Reproducibility

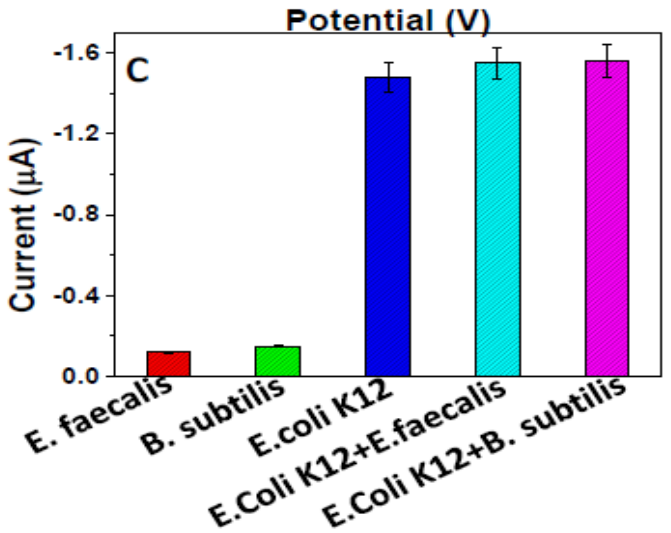
- investigated by repeating with five different electrode

1- unique preparation of the label

2- immunosensor construction methods

c. Stability

- Similar test was also performed
- stored in the refrigerator at 4 ºC.



Conclusions

- A simple method was proposed to disperse AuNPs on PPy surface uniformly.
- A highly sensitive sandwiched E.coli K12 immunosensor was constructed by using PPy-rGO/AuNPs as signal amplifier and PPy@Fc/AuNPs as signal generator.
- electrochemical biosensors offer unique opportunities by

high sensitivity	potential miniaturization
good selectivity	Reproducibility
wide linear range	low cost

- Testing of the sensor in real-to-life media (city water and milk) demonstrates that this approach is useful and should allow us to further develop this sensor into a practical device.

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Gold nanoparticles-based multifunctional nanoconjugates for highly sensitive and enzyme-free detection of *E.coli* K12

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