بسم الله الرحمن الرحیم

Polyaniline-pectin nanoparticles immobilized paper based colorimetric sensor for detection of *E.coli* in milk and milk products

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Title

- Introduction
- Methods
- Results
- Conclusion

Introduction

- Milk and milk products play an important role in human diet.
- *E.coli* is One of the frequently microorganisms in milk and milk products.
- Rapid detection of *E. coli* is a major challenge faced by food processing industries.
- Traditionally used conventional methods for the detection of *E. coli* culture-based.

Colorimetric sensor using Polyaniline-Pectin nanoparticles

- Detection approaches using paper-based colorimetric sensors: simplicity, selectivity, rapidity and cost effectiveness
- The developent of a simple and cost-effective paper based colorimetric sensor using Polyaniline-Pectin nanoparticles (PANI- PEC NPs).

Methods

Synthesis of PAni- Pec nanoparticles

APS solution

Aniline monomer+ pectin+ Hcl in milli-Q water

Stirring for 4h RT

PAni-Pec dispersion

Precipitation with 50% ethanol

Synthesis of PAni- Pec nanoparticles

filtration

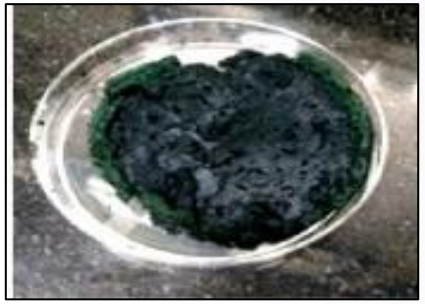
Washing with 1:1mixture of ethanol-water

Pure Pani-pec precipitate

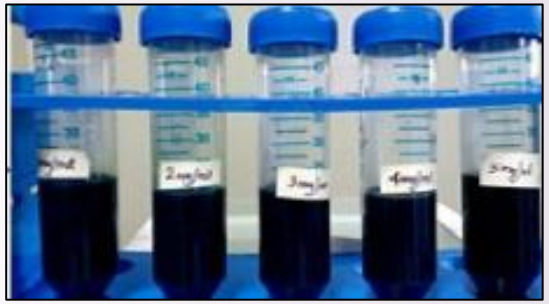
Lyophilization

Synthesis of PAni- Pec nanoparticles

Lyophilisation at -84±1ºC , 1±0.5 torr,4h



Lyophilized Pani-pec dispersion



1-5mg/ml solution of Pani-pec in Milli-Q water

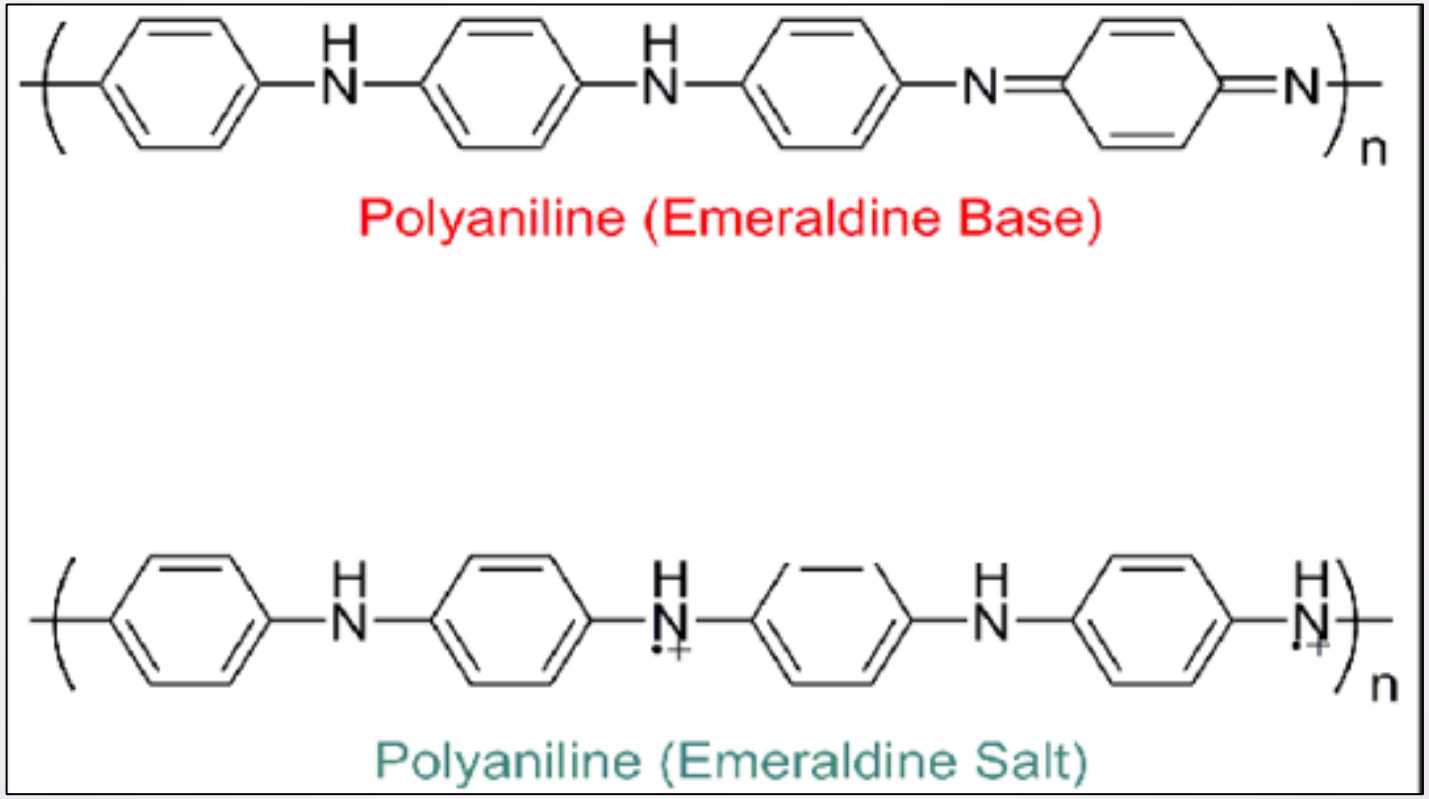
Ultra sonication for 4h

ES form of PANI-PEC solution

NaoH 1N

EB form of PANI-PEC solution

Form EB & ES polyaniline

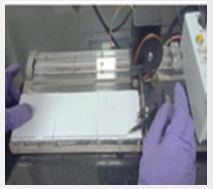


Characterization of PANI- PEC nanoparticles

- FTIR spectrum : range 4000–400 cm− 1
- UV–Vis spectrum : range 300–1000 nm
- Zetasizer nano

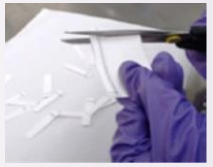
Construction of colorimetric sensor strips

1. Fabrication of PAni-pec solution(1-5mg/ml)

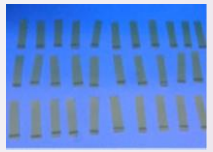


2. Drying at 45˚C/30 min in incubator

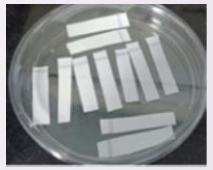
3. Cutting into strips



4. Exposure to UV in 20 min for surface sterilization



5. PAni-pec strips



Optimization of media components

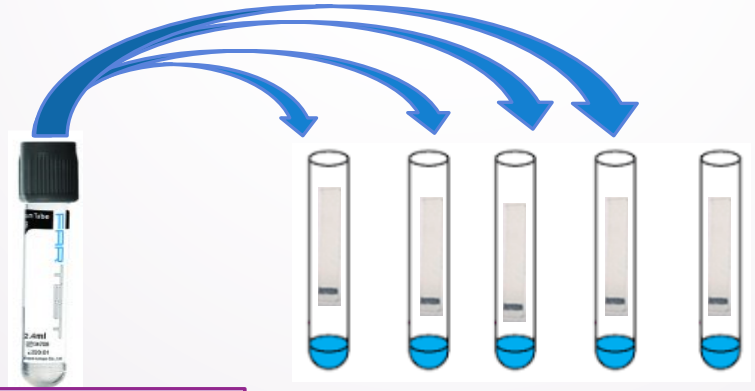
- Environmental components with different concentrations(0.1-5%) were optimized for *E. coli* selection:

lactose, tryptophan, yeast extract, chondroitin sulphate, sodium lauryl sulphate, potassium chloride, tergitol-7, gentamycin sulphate and ampicillin trihydrate.

- Preparation Cell suspensions of different cell levels (~8–~0.5 log CFU/ mL) of *E. coli* in normal saline and inoculated into media prepared.
- ✓ Detection of *E.coli* with maximum growth in minimum time .

Optimization of sample volume

- Prepare ~0.5 log CFU/mL *E. coli* in normal saline .
- Inoculation of different volumes of normal saline into the optimal medium.
- Rapid color change ➡ Optimal sample volume
- Volume optimized: 500 µL



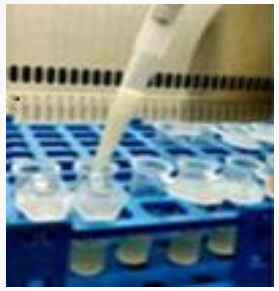
Log 0.5 Cfu/ml

Detection of *E. coli* using PANI- PEC colorimetric sensor

- Preparation of *E.coli* suspension with different concentrations of 8 log CFU/mL to ~0.5 log CFU/mL .
- Evaluated change coloration within 30 min to 12 h.
- Perform protocols for raw milk, pasteurized milk .

Protocol for detection of *E. coli* using paper strip sensor

0.5 ml optimized media

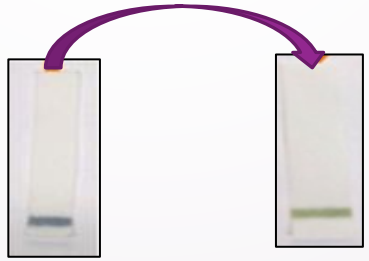


Addition of 0.5 ml of sample

Vortex

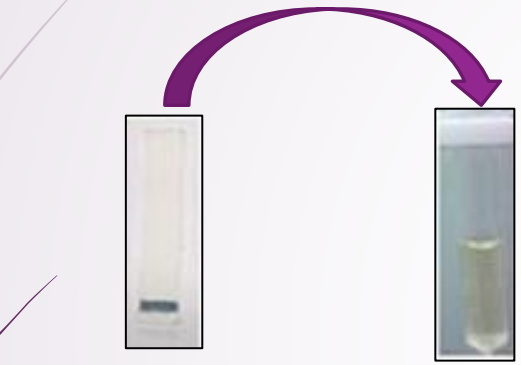
Immersion of PAni-Pec strip

Incubation at 37˚C



Color change from blue to green indicates the presence of *E.coli*

Mechanism of detection *E. coli*



PAni-Pec sensor strips(Blue)

Selective media

In absence of *E.coli*

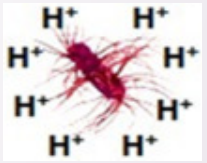
Blue

In presence of *E.coli*

Green

Metabolism

Release of acidic end products



PAni in EB form

H^+

Proton doping of PAni

PAni in ES form

Selectivity study of PANI- PEC colorimetric strip-based sensor

- Determination of sensor selectivity with gram-positive and gram-negative bacteria :
- *Listeria monocytogenes* / *Staphylococcus aureus* / *Bacillus cereus* / *Enterococcus faecalis*
- *Salmonella arizonae*/ *Enterobacter aerogenes* / *Shigella flexnerii*

Citrobacter freundii / *Yersinia enterocolitica* / *Proteus vulgaris*

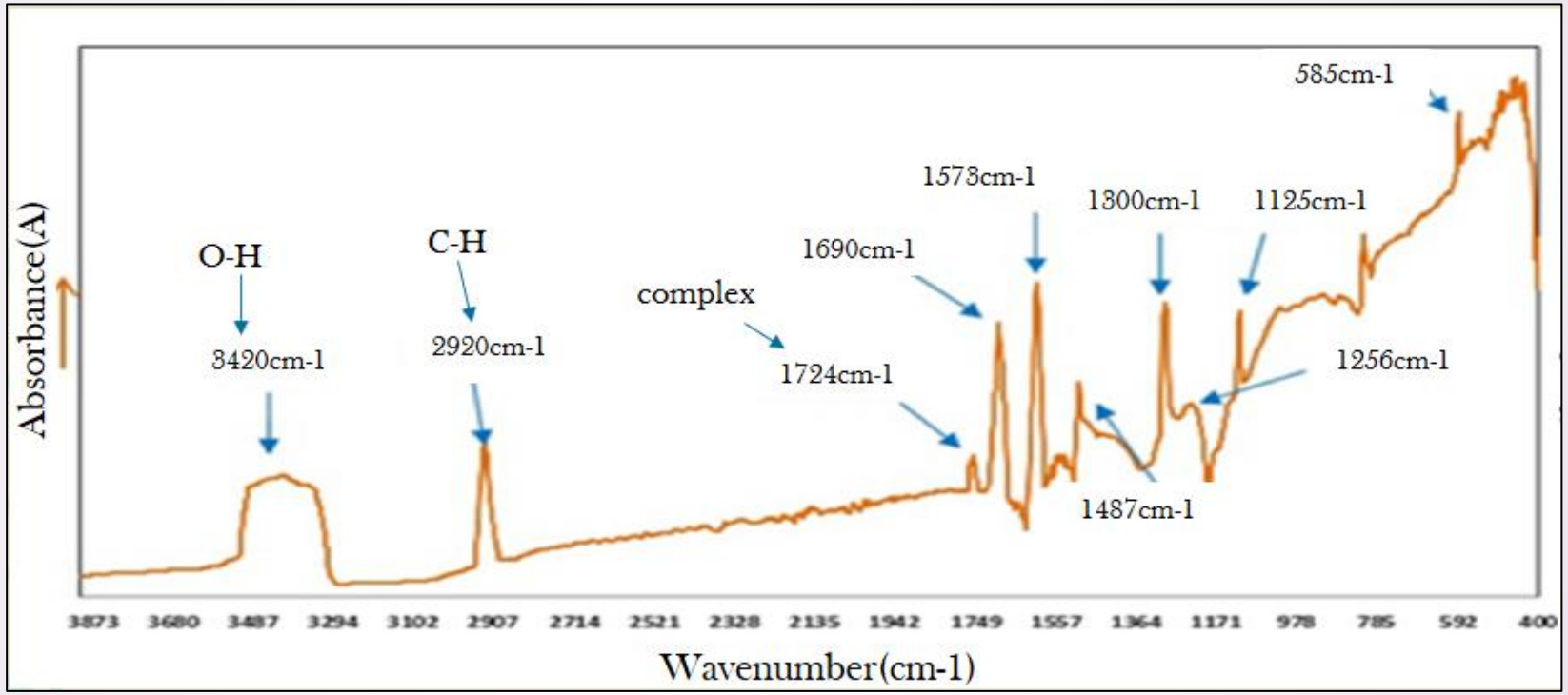
Klebsiella pneumoniae / *Serratia marcescens*

Shelf-life study of PANI- PEC colorimetric strip

- Store the strips at room temperature, 4 °C and- 20°C.
- Check the sensitivity and intensity of the color for 6 months at intervals of 15 days.

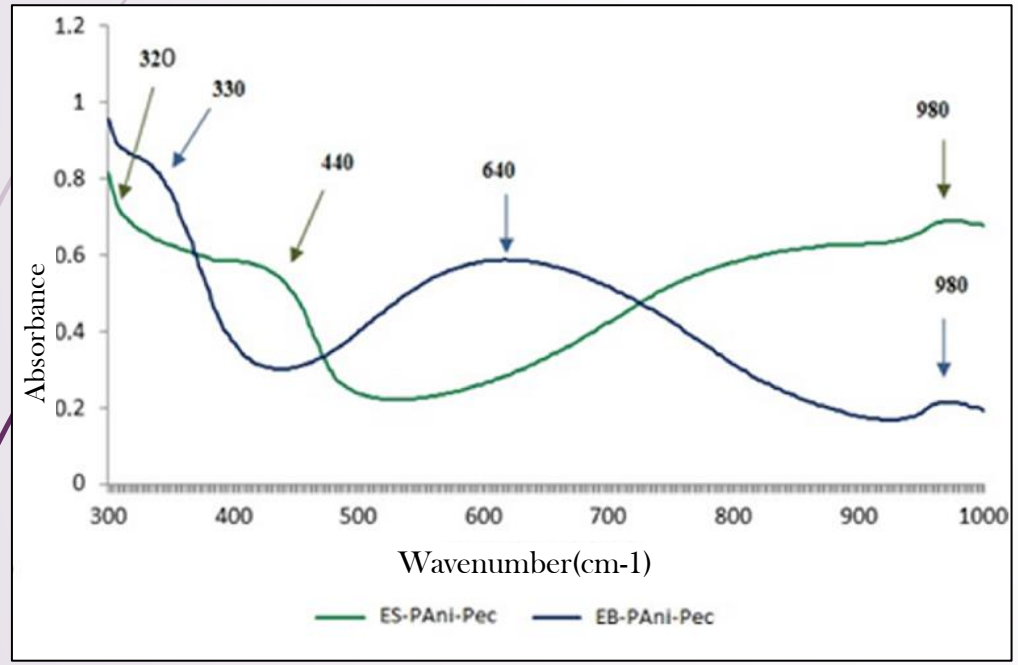
Results

characterization of PANI- PEC nanoparticles

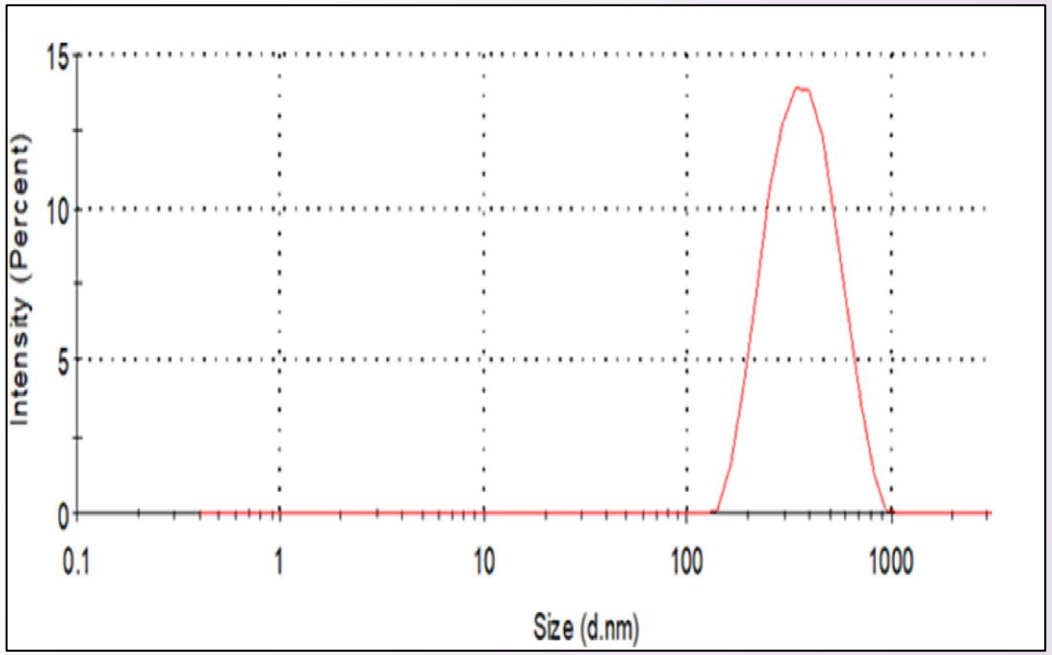


FTIR spectroscopy of PANI- PEC nanoparticles

characterization of PANI- PEC nanoparticles



UV-Vis absorption spectra

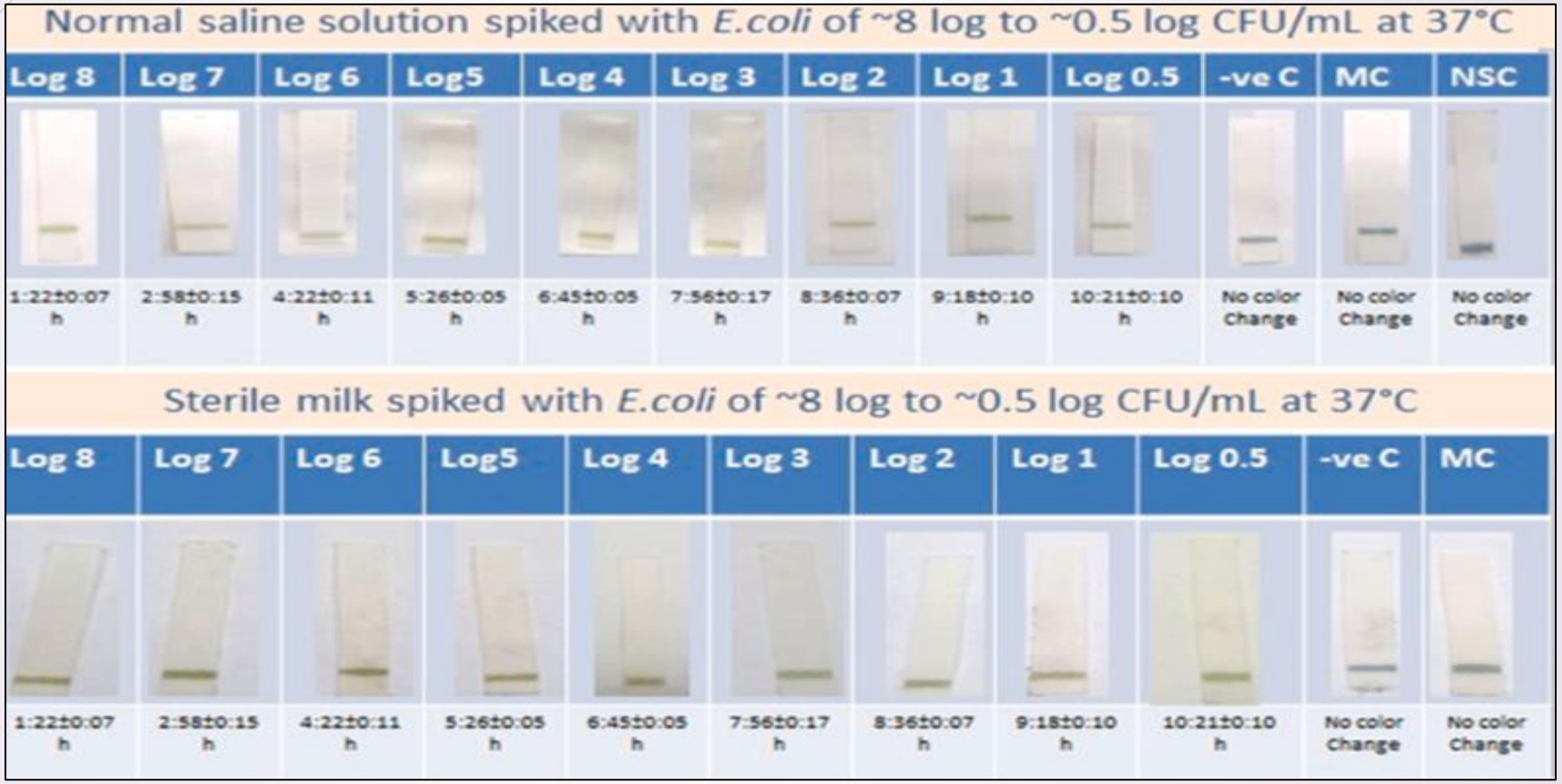


Size distribution profile

Construction of colorimetric sensor strips

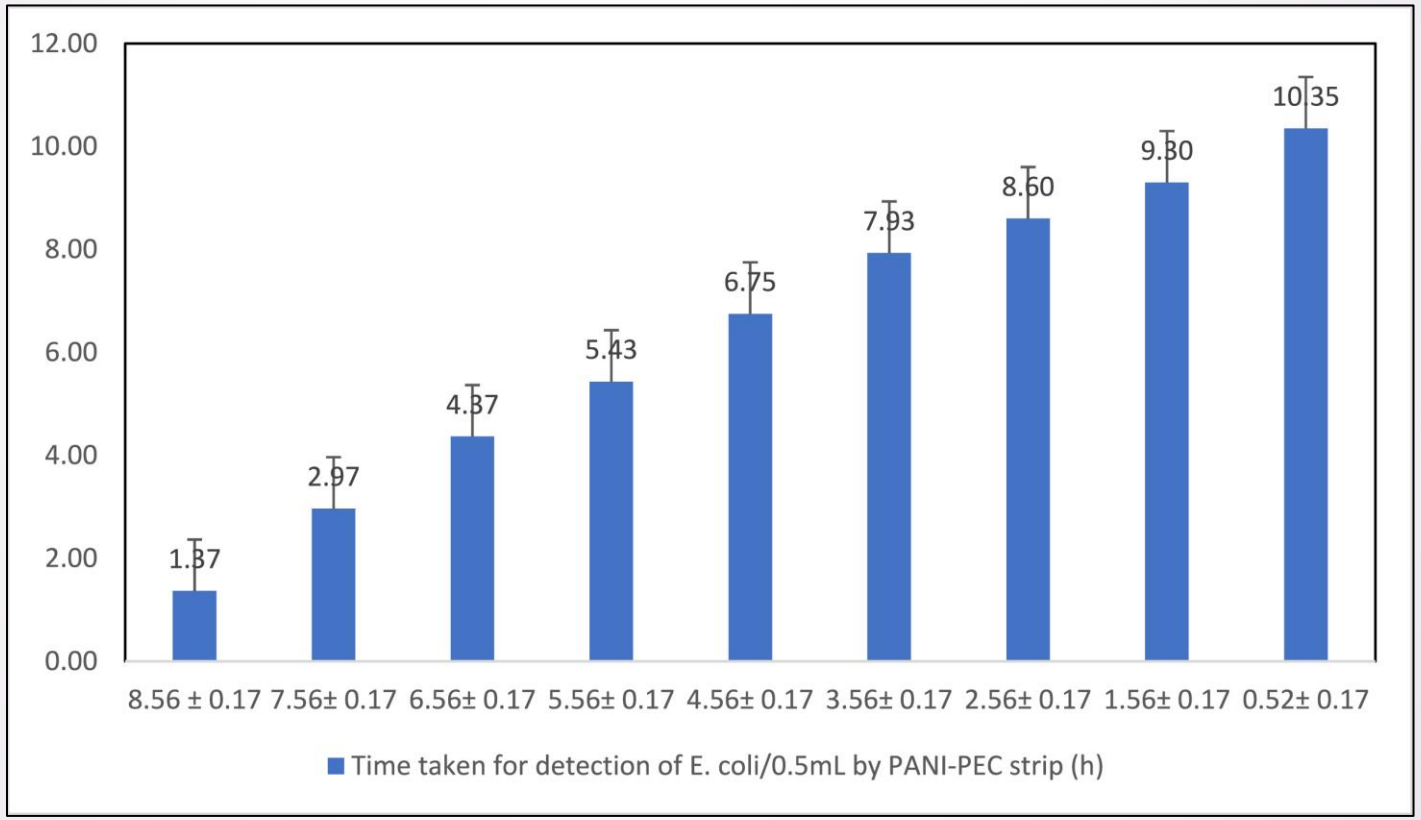
Optimization of PANI-PEC nanoparticles concentration.

Detection of *E. coli* using optimized PANI- PEC colorimetric sensor



Sensitivity of PANI-PEC paper strip for the detection of *E. coli.*
(-ve C -:Negative control; MC:Media control; NSC: Normal saline control).

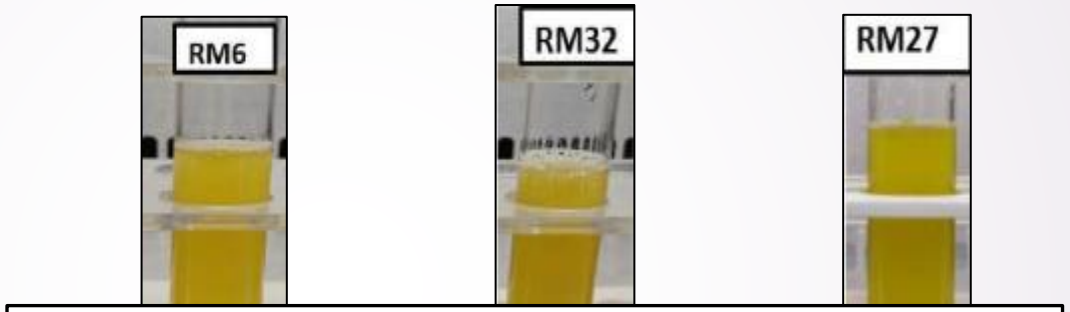
Detection of *E. coli* using optimized PANI- PEC colorimetric sensor



Plot showing sensitivity of PANI-PEC strip based sensor assay for detection of *E. coli*

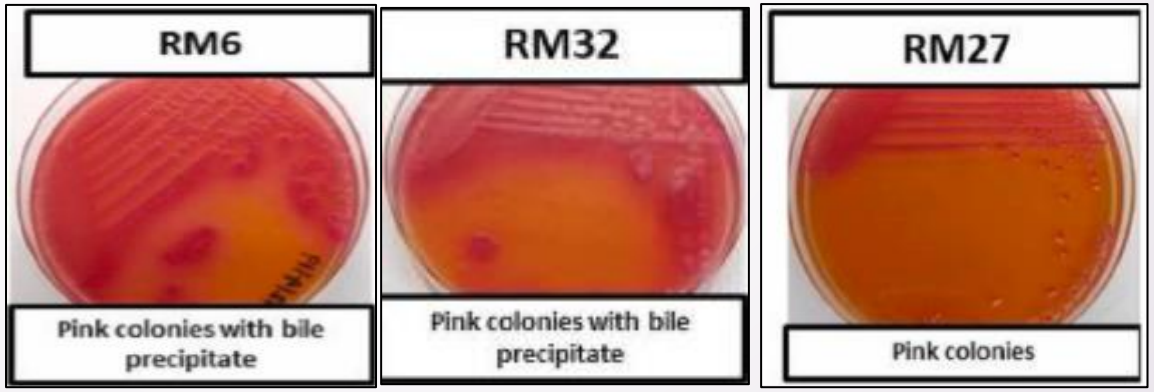
Detection of *E. coli* using optimized PANI- PEC colorimetric sensor

Enrichment in MacConkey broth



Yellow reaction with acid and gas production

Streaking on MacConkey agar



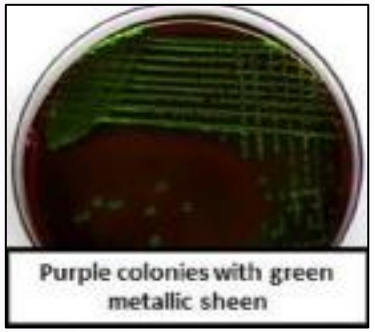
IS: 5887 (Part-1):1976 method

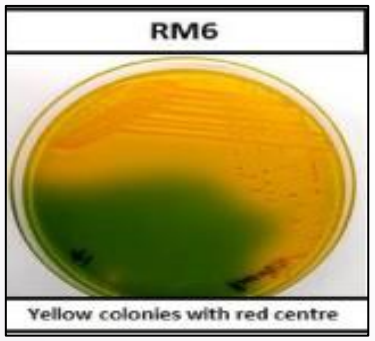
Detection of *E. coli* using optimized PANI- PEC colorimetric sensor

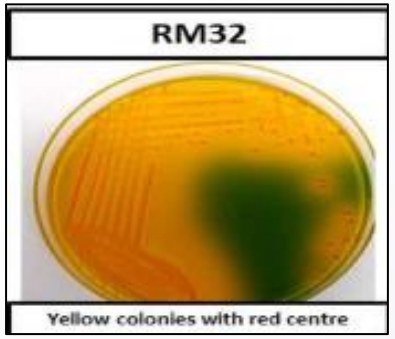
Streaking on EMB agar

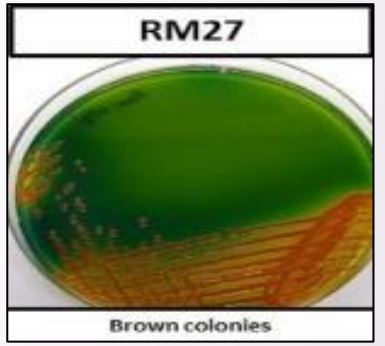
Streaking on Tergitol-7 agar

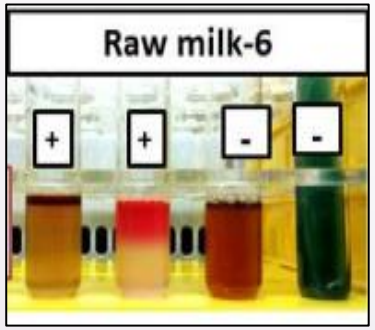
IMVIC

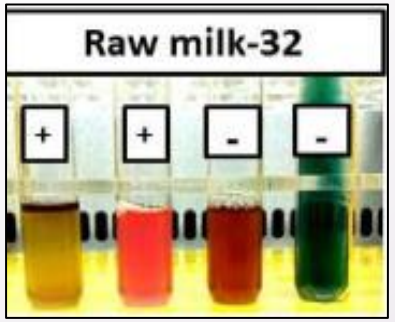


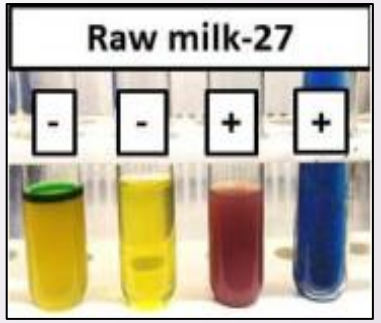












Shelf-life study of PANI- PEC colorimetric strip

Month	Log CFU/mL	Room temperature		4°C		-20°C		Freshly made strips		Detection time
		Log 8	Control	Log 8	Control	Log 8	contol	Log 8	control	
2	8.34									1h 30 min
		Log 8	Control	Log 8	Control	Log 8	contol	Log 8	control	
4	8.74									1h 15min
		Log 8	Control	Log 8	Control	Log 8	contol	Log 8	control	
6	8.52									1h 21 min

Sensitivity of PANI-PEC colorimetric strips stored at different temperatures

Conclusion

- The developed PANI- PEC colorimetric strip-based sensor assay is simple, sensitive and selective. For this reason, it has good sensitivity and selectivity for identifying *E. coli* without interfering with factors like physico-chemical properties of milk.
- Absence of any bio-recognition elements such as antibody, DNA or any enzymes in the strips along with the excellent thermal and environmental stability of polyaniline dramatically improves its shelf life.

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Polyaniline-Pectin nanoparticles immobilized paper based colorimetric sensor for detection of *Escherichia coli* in milk and milk products

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Thank
You

Optimization of media components &sample volume

- Optimization medium composition:

lactose (0.9%), tryptophan (0.1%),yeast extract (0.45%), chondroitin sulphate (0.015%), sodium lauryl sulphate (0.1%),potassium chloride (2%),tergitol-7 (0.0125%), gentamycin sulphate (0.00016%) and ampicillin trihydrate (0.015%).

- The sample volume for the assay was optimized to 500 μL.

Selectivity study of PANI- PEC colorimetric strip-based sensor

Gram negative contaminants	Log CFU/mL
Salmonella arizonae	5.4 ± 0.12
Enterobacter aerogenes	5.2 ± 0.12
Shigella flexneri	7.4 ± 0.12
Citrobacter freundii	6.3 ± 0.76
Yersinia enterocolitica	6.5 ± 0.15
Proteus vulgaris	7.6 ± 0.11
Serratia marcescens	5.7 ± 0.07

Inhibition level of Gram negative contaminants in the developed assay