Chapter V

Discussion and Comparative Analysis

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DISCUSSION AND COMPARATIVE ANALYSIS

- *This chapter provides a comparative study between the current work and previously reported studies in the literature.*
- *A comparative evaluation of all sensing architectures introduced in the thesis has also been studied.*
- *Special emphasis has been made on the advancements and contributions made by the current study.*

5.1 Comparative analysis of colorimetric sensing architectures

Colorimetric sensors, known for their simplicity and visual readout, have evolved significantly over the years. Each sensing architecture offers distinct advantages such as heightened sensitivity, selectivity, and ease of use. In the previous chapters, various colorimetric sensing architectures have been implemented to assess milk adulteration and contamination. As detailed in those chapters, all these sensors are equipped with their own merits and demerits. To get a better insight into these implemented structures, a comparative study often helps. It highlights the strengths and limitations of each approach, while showcasing the innovations introduced in the current study. Accordingly, this section is devoted in bringing out comparative analysis of the developed colorimetric sensing architectures devised in this thesis work against the existing architectures.

In this regard, various studies for colorimetric sensing have been reviewed, and a comparative overview is provided alongside the relevant works reported in the literature for colorimetric sensing (**Table 5.1**; **Table 5.2**; **Table 5.3**; and **Table 5.4**).

Table 5.1: *Comparative study of melamine sensing by colorimetric method.*

Sensing Element	Reducing agent	Functionalising agent	Selectivity	Recovery	Limit of Detection/ Lowest limit of sensing	References
TSC-AuNPs	Trisodium citrate (TSC)	TSC	Glucose, hydrogen peroxide, urea, formalin, sucrose dextrose, nitrate	95%-105%	0.05 ppm	[1]
MTT-AuNPs	TSC	1-(2-mercaptoethyl)-1,3,5-triazinane-2,4,6-trione (MTT)	Cytosine, uracil, and thymine	Not performed	2.5 ppb	[2]
TSC-AgNPs	Sodium borohydride ($NaBH_4$)	TSC	Vitamin C, lactose, glucose, polypeptide, NH^{4+}, Na^+, K^+, Ca^{2+}, SO_4^{2-}, NO^{3-}, Cl^-	88.83% - 114.29%	0.29 ppm	[3]
SFA-AgNPs	$NaBH_4$	Sulfanilic acid (SFA)	Lysine, tryptophan, methionine, leucine, isoleucine, phenylalanine, valine, NO^{3-}, pyrophosphate, citrate, CO_3^{2-}, EDTA, Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{3+}, Na^+, glucose, fructose, sucrose	103% -109%	1.34 ppb	[4]
AA-AgNPs	Ascorbic acid (AA)	AA	Dextrose, glycine, leucine, citric acid, lactose, zinc, sodium	Not performed	0.1 ppm	[5]
CT/TPP-AuNPs	Chitosan (CT) and tripolyphosphate (TPP)	CT	Ca^{2+}, Zn^{2+}, Fe^{3+}, Mg^{2+}, K^+, Na^+, glucose, urea, sucrose	85% - 107%	6 ppb	[6]
GA-AgNPs	Gallic acid (GA)	GA	Serine, phenyl alanine, alanine, tryptophan, arginine, isoleucine, glycine, leucine, vitamin B6, tyrosine, urea	97.1-102.6%	0.456 ppb	[7]
B-CDT-AgNPs	β-cyclodextrin (β-CDT)	β-CDT	Alanine, citric acid, glucose, glycine, thymine, cysteine, histidine, NaCl, adenine, NH^{4+}, urea, lactase	80.5–109.02%	0.628 ppm	[8]
p-NA-AgNPs	$NaBH_4$	p-nitroaniline (NA)	Na^+, $H_2PO_4^-$, 2-aminoethanesulfonic acid, HPO_4^{2-}, Cl, Ca^{2+}, Zn^{2+}, phenylalanine, Fe^{3+}, glucose, threonine, valine, leucine	Not performed	0.1 ppm	[9]
DP-AgNPs	Dopamine (DP)	DP	Phenylalanine, DL-leucine, L-glutamate, sulfanilic acid, Mg^{2+}, galactose, lysine, urea, glucose	98.5%	10 ppb	[10]
JG-AgNPs	Jatropha gossypifolia (JG)	JG	Cyanuric acid	96- 122 %	252 ppb	[11]
TA-AgNPs	Tannic acid (TA)	TA	Urea, glucose, glycine, ascorbic acid	99.5–106.5%	1.26 ppb	[12]
PH-AgNPs	. Caffeic acid (CA)	Parthenium histerophorus (PH)	Lactose, citric acid, cysteine, lysine, magnesium, dextrose, leucine, glycine	96%	0.5 ppm	[13]
GT- AgNPs (Liquid based)	**Green Tea (GT)**	**GT**	**Urea, formalin, salicylic acid, dextrose, cyanuric acid**	**93%**	**1.44 ppm**	**Reported Work**
MA- AgNPs (Paper Based)	**$NaBH_4$**	**Maleic acid (MA)**	**Hydrogen peroxide, formalin, lead, arsenic, cadmium, mercury**	**Not performed**	**0.76 ppm**	**Reported Work**

Table 5.2: *Comparative study of hydrogen peroxide sensing by colorimetric method.*

Sensing Element	Reducing agent	Functionalising agent	Selectivity	Recovery	Limit of Detection/ Lowest limit of sensing	References
CW-AgNPs	Cellulose nanowhiskers (CW)	CW	Cu^{2+}, Zn^{2+}, Fe^{2+}, uric acid, Glucose	98 %	0.476 ppb	[14]
SC-AgNPs	Sericin (SC)	SC	Ascorbic acid, dopamine, phenylalanine, glutamate, tryptophan, cysteine, valine, isoleucine, glycine, lysine, histidine, asparagine, leucine, fructose, lactose, maltose, sucrose, glutathione, human serum albumin, urea, uric acid, catechol, calcium chloride, potassium chloride, sodium chloride, magnesium chloride	Not performed	0.15 ppm	[15]
SB-AgCl NPs	Sargassum Boveanum (SB)	SB	Interfering cations and anions	98.58-100.80%	0.29 ppb	[16]
BH-AgNPs	Benincasa hispida fruit extract (BH)	BH	Not performed	Not performed	34.014 ppm	[17]
CT-AgNPs (Without substrate)	**Cotton Leaves (CT)**	**CT**	**Formalin, melamine, salicylic acid, urea, ammonium sulphate**	**92%**	**8.46 ppm**	**Reported Work**
AgNPs	**Green Tea (GT)**	**-**	**Melamine, formalin, lead, arsenic, cadmium, mercury**	**Not Performed**	**5.60 ppm**	**Reported Work**

Table 5.3: *Comparative study of formalin sensing by colorimetric method.*

Sensing Element	Reducing agent	Functionalising agent	Selectivity	Recovery	Limit of Detection/ Lowest limit of sensing	References
TL-AuNPs	TSC	Tollen's reagent (TL)	Not performed	Not performed	100 ppm	[18]
ATP-AgNCs (Silver Nanocubes)	Sodium sulfide and Poly Vinyl Pyrollidone (PVP)	4-aminothiophenol (4-ATP)	Acetaldehyde, benzaldehyde, acetone, Glucose, sucrose	Not performed	1.14 ppm	[19]
RSC-AuNPs	TSC	Resorcinol (RSC)	Acetone, acetaldehyde, glyoxal, butanal, benzaldehyde, glucose	Not performed	0.5 ppm	[20]
L-Cyst-AgNPs	**$NaBH_4$**	**L-Cysteine (L-Cyst)**	**Melamine, urea, hydrogen peroxide, dextrose**	**90%**	**3.51 ppm**	**Reported Work**
L-Cyst-AgNPs	**$NaBH_4$**	**L-Cyst**	**Melamine, hydrogen peroxide, lead, arsenic, cadmium, mercury**	**Not performed**	**4.53 ppm**	**Reported Work**

Table 5.4: *Comparative study of salicylic acid sensing by colorimetric method.*

Sensing Element	Reducing agent	Functionalising agent	Selectivity	Recovery	Limit of Detection/ Lowest limit of sensing	References
GE-SeNPs (Selenium Nanoparticles)	Garlic extract (GE)	GE	Not performed	Not performed	138.12 ppm	[21]
WR-AgNPs	**Watermelon Rind (WR)**	**WR**	**Melamine, Urea, Formalin**	**96%**	**0.55 ppm**	**Reported Work**

Compared to previously reported studies, particularly those focused on melamine, hydrogen peroxide, formalin and salicylic acid detection in milk, our work demonstrates several key advantages; such as the method that we have demonstrated is facile and eco-friendly. It is more straightforward, robust, and environmentally sustainable compared to the chemical-intensive techniques used in prior studies. Our method offers higher accuracy and recovery rates, surpassing that of other reported methods, ensuring more reliable quantification of these adulterants. It also offers a low LOD. The LOD achieved in our study is just below the permissible limits set by various governmental regulatory bodies, making it safer for practical application. Moreover, our approach is highly selective, allowing precise detection of each adulterant even in the presence of other commonly added milk adulterants, whereas the other methods exhibited interference. Most importantly, the reported method is highly cost-effective and thus is scalable. Since our method is less resource-intensive, it can be utilised for widespread monitoring and sensing of the adulterants in milk.

The detection of milk contaminants is seldom addressed in the existing literature, with most studies primarily focusing on the detection of milk adulterants. However, the presence of toxic contaminants in milk can also lead to severe health risks, necessitating their timely detection to prevent potential health hazards. While few methods for detecting heavy metals (HM)—the most common milk contaminants—have been reported, these studies often focus on detection in water rather than in milk. Milk, being a complex matrix, poses significant challenges for the detection of contaminants due to its' intricate composition. Therefore, there is a pressing need for robust, simple, and facile methods specifically tailored for detecting contaminants directly in milk. **Table 5.5** provides a comparative overview of all the works related to detection of contaminants reported in literature with the current work.

Table 5.5: *Comparative study of contaminant sensing by colorimetric method.*

Sensing Element	Sensing Analyte	Reducing agent	Functionalising agent	Sensing Time	Limit of Detection/ Lowest limit of sensing	References
CA-AuNPs (Without substrate)	Mercury in milk	TSC	Cysteamine (CA)	Not mentioned	6.02 ppb	[22]
PP-AuNPs (Without substrate)	Mercury in milk	TSC	Papain (PP)	Not mentioned	1.0 ppm	[23]
CT-Au/Pt NCs (nanoclusters) (Without substrate)	Lead in milk	$NaBH_4$	CT	Not mentioned	3.32 ppb	[24]
L-Cyst-AuNPs (Without substrate)	Cadmium in milk	TSC	L-Cysteine	Not mentioned	10 ppm	[25]
PVA/BRB-AgNPs (Paper Based)	**Mercury in milk**	**Banana Root Bulb (BRB)**	**Poly Vinyl Alcohol (PVA)**	**1 min**	**0.87 ppm**	**Reported Work**
CA-AgNPs (Paper Based)	**Arsenic in milk**	**$NaBH_4$**	**Citric Acid (CA)**	**1 min**	**0.65 ppm**	**Reported Work**
L-Glu-AgNPs (Paper Based)	**Lead in milk**	**$NaBH_4$**	**L-Glutamine**	**2 min**	**0.35 ppm**	**Reported Work**
SA-AgNPs (Paper Based)	**Cadmium in milk**	**$NaBH_4$**	**Salicylic Acid (SA)**	**1 min**	**0.73 ppm**	**Reported Work**

As compared to previously reported studies on the detection of HM as contaminants in milk, our work offers a more efficient and facile approach for identifying a broad range of contaminants using a single paper-based platform. This platform is selectively impregnated with functionalized NPs, allowing targeted and specific detection of multiple contaminants in one streamlined process. Our method also achieves a significantly lower detection time, making it more practical for real-time sensing applications. In addition, we report a low LOD, which enhances its sensitivity ensuring highly accurate qualitative estimation of presence of contaminants in milk. The sensitivity and selectivity reported in our study surpasses that of previous techniques, providing a more reliable safeguard against potential health risks. Furthermore, the fabrication of our sensing setup is achieved through a cost-effective and scalable process, in contrast to many earlier methods that require complex, resource-intensive protocols. The combination of simplicity, rapid response, high sensitivity, and affordability makes our approach highly suitable for routine milk quality monitoring and offers a substantial improvement over existing technologies.

5.2 Comparative analysis of LSPR-based sensing architectures

Colorimetric methods are often employed as a qualitative tool for sensing of adulterants and contaminants in milk. However, to ensure accurate quantification of each adulterant, a more precise technique is required—one capable of detecting even trace amounts. This level of sensitivity can only be achieved using LSPR-based plasmonic nanofilms. These nanofilms detect subtle changes in the refractive index of the medium surrounding the NPs, enabling selective and precise quantification of each adulterant in milk.

Table 5.6 and **Table 5.7**, provides a comprehensive comparison of the reported work with the studies previously reported in the literature, highlighting the advantages of the present LSPR-based approach for quantifying milk adulterant.

Table 5.6: *Comparative study of melamine sensing by LSPR method.*

Sensing Element	Reducing agent	Functionalising agent	Selectivity	Recovery	Limit of Detection/ Lowest limit of sensing	References
p-NA-AuNPs (Cuvette based)	TSC	p-NA	Cyanuric acid, uracil, urea, m-phenylenediamine.	Not performed	0.01 ppb	[26]
TSC-AuNPs (Optical Fibre based)	TSC	TSC	Not performed	99.2%~111%	4.16 ppb	[27]
MA-AuNPs (Cuvette based)	**$NaBH_4$**	**MA**	**Formalin, urea, ammonium sulphate, hydrogen peroxide, salicylic acid, cyanuric acid**	**96%-99.4%**	**10.48 ppb**	**Reported Work**

Table 5.7: *Comparative study of hydrogen peroxide sensing by LSPR method.*

Sensing Element	Reducing agent	Functionalising agent	Selectivity	Recovery	Limit of Detection/ Lowest limit of sensing	References
PVA-OT-AgNPs	**Ocimum tenuiflorum (OT) Leaves**	**PVA**	**Formalin, urea, melamine, dextrose, salicylic acid, ammonium phosphate**	**97%-108%**	**2.72 ppb**	**Reported Work**

By comparing the work presented in this thesis with previous studies, we found that no protocols have been reported for the LSPR-based sensing of hydrogen peroxide using nanofilm fabrication, highlighting a novel aspect of our approach. Additionally, while some existing methods for melamine detection utilize gold nanoparticles (AuNPs), which significantly increase the cost, our detection method is both more cost-effective and facile. Our approach offers several advantages over previously reported methods: the fabrication process for our sensor is simpler, making it scalable and suitable for real-time monitoring of adulterants, even in trace amounts. Our method presents a practical and accessible alternative for milk adulteration monitoring, combining cost-effectiveness and ease of use while addressing the limitations of the complex and expensive techniques commonly reported in the literature.

5.3 Comparative analysis of electrochemical sensing architectures

Electrochemical sensing offers several distinct advantages over LSPR and colorimetric sensing methods, making it a more effective tool for detecting various analytes. Unlike LSPR, which relies on changes in the refractive index, and colorimetric methods that provide primarily qualitative results, electrochemical sensors deliver highly sensitive, quantitative measurements. Electrochemical techniques can detect even trace amounts of analytes with greater precision, ensuring higher accuracy.

Moreover, electrochemical sensors typically have faster response times, lower detection limits, and can operate in complex matrices like milk, where other methods might struggle. These sensors are also highly adaptable, allowing for the detection of multiple substances through simple modifications. Their scalability, portability, and potential for real-time monitoring further enhance their utility, making electrochemical sensing a more robust and versatile platform for detecting contaminants and adulterants in challenging environments. **Table 5.8**, **Table 5.9**, and **Table 5.10** provides a comparison overview of the electrochemical sensing of milk adulterants presented in the thesis with that existing in the literature.

Table 5.8: *Comparative study of urea sensing by electrochemical method.*

Sensing Element	Recovery	Limit of Detection/ Lowest limit of sensing	References
AgNP-coated electrode	89.38%	8.41 ppm	[28]
TiO_2-LL@AgNPs	Not performed	0.149 ppm	[29]
APTES/GT-AuNPs/APTES coated ITO glass slide	**95.05%- 99.44%**	**4.02 ppm**	**Reported Work**

Table 5.9: *Comparative study of melamine sensing by electrochemical method.*

Sensing Element	Recovery	Limit of Detection/ Lowest limit of sensing	References
Reduced graphene oxide (r-GO) modified copper nanoflowers (NFs) modified glassy carbon electrode (GCE)	87.76 - 90.43%	0.63 ppb	[30]
PEG/MA-AgNPs/APTES coated ITO glass slide	**97.73%-101.72%**	**12.24 ppb**	**Reported Work**

Table 5.10: *Comparative study of hydrogen peroxide sensing by electrochemical method.*

Sensing Element	Recovery	Limit of Detection/ Lowest limit of sensing	References
AuPt NPs modified electrode	82-116%	0.085 ppm	[31]
Bimetallic Pd-Ag NPs functionalized r-GO	96.76- 102.42%	23.81 ppb	[32]
AgNPs-modified r-GO	97.20-99.41%	24.83 ppb	[33]
PVP/AgNPs/APTES coated ITO glass slide	**98.73%-100.45%**	**5.19 ppb**	**Reported Work**

In comparing the work presented in this thesis with previous studies, we found that very few protocols have been reported for electrochemical sensing of adulterants using plasmonic NP-based electrodes, underscoring the novelty of our approach. Moreover, existing methods for detecting urea, melamine and hydrogen peroxide often rely on expensive and complex materials, which significantly increase the overall cost of fabrication. In contrast, our fabrication approach for electrochemical detection is not only more cost-effective but also significantly simpler, providing an accessible and efficient solution for accurate adulterant detection.

Our approach provides several advantages over previously reported methods: the fabrication of our electrochemical sensor is simpler, enabling scalability and making it ideal for real-time monitoring of adulterants, even at trace levels. This combination of affordability, ease of fabrication, and high sensitivity positions our electrochemical sensing technique as a practical and efficient solution for widespread use in milk adulterant sensing applications. It offers a significant improvement over the more costly and complex techniques described in the existing literature.

5.4 Overall comparison of reported sensing methods

This work primarily focuses on three distinct approaches for the detection of adulterants, each with its own methodology and advantages. The first approach is colorimetric sensing, which is subdivided into two categories: liquid-based colorimetric detection and paper-based colorimetric detection. The second approach involves LSPR-based sensing, and the third is electrochemical sensing. Each of these sensing techniques presents unique advantages and limitations, making them suitable for different applications and conditions. For instance, colorimetric methods offer simple and rapid visual detection, while LSPR-based sensing provides enhanced sensitivity for detecting any changes in the external surroundings of the NPs. Electrochemical sensing, on the other hand, offers highly accurate, quantitative detection and is well-suited for real-time monitoring of adulterants, even in complex matrices such as milk.

Table 5.11 provides an overall comparison of the sensing parameters for all devices reported in this thesis, underscoring the advantages of each sensing architecture.

This comparison highlights how each method can be strategically applied depending on the specific requirements of real-time adulterant detection, offering insights into their performance, sensitivity, and practicality in various scenarios.

Table 5.11: *Comparative analysis of the sensing performance of all the present reported sensing architectures.*

Mode of Sensing	Analyte Detected	Limit of Detection	Cost-Effectiveness	Instrument Required	Advantage
Colorimetric (Liquid Based)	Melamine	1.44 ppm	Highly Cost effective	Naked Eye	Can be used for onsite sensing of real samples without any complicated sample pretreatment
	Hydrogen Peroxide	8.46 ppm			
	Formalin	3.51 ppm			
	Salicylic acid	0.55 ppm			
Colorimetric (Paper substrate based)	Melamine	0.76 ppm	Highly Cost effective	Naked Eye	Higher portability, ease of use, and requires less sample volume, hence, more practical for on-site and real-time detection
	Hydrogen Peroxide	5.60 ppm			
	Formalin	4.53 ppm			
	Mercury	0.87 ppm			
	Arsenic	0.65 ppm			
	Lead	0.35 ppm			
	Cadmium	0.73 ppm			
LSPR Method	Melamine	10.48 ppb	Less cost-Effective compared to colorimetric method	UV-Vis Spectrophotometer	Higher sensitivity, allows precise and selective detection of adulterants, even at extremely low concentrations with faster response times
	Hydrogen peroxide	2.72 ppb			
Electrochemical Method	Urea	4.02 ppm	Less cost-Effective compared to colorimetric and LSPR method	Electrochemical Station	Greater quantitative accuracy, lower detection limits, and faster response times
	Melamine	12.24 ppb			
	Hydrogen peroxide	5.19 ppb			

For qualitative analysis, colorimetric methods are often favoured due to their simplicity, rapid visual readout, and ease of use, making them ideal for quick, and preliminary detection of adulterants. In contrast, for more precise quantitative estimation, LSPR-based methods are preferred, as they offer high sensitivity to changes in the refractive index and enable accurate measurements of analyte concentrations. When it comes to detecting trace amounts of adulterants, electrochemical sensing schemes are the most effective, providing superior sensitivity, lower detection limits, and the ability to quantify even minute concentrations of adulterants in complex matrices. Thus, the choice of sensing method depends on the specific need for either qualitative or quantitative analysis and the required level of sensitivity.

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