Chapter VI

Conclusion and Future Prospects

CHAPTER VI

CONCLUSION AND FUTURE PROSPECTS

- *This chapter summarizes the key findings of the thesis and discusses all reported works.*
- *Additionally, it outlines prospects for future investigations based on the presented work.*

6.1 Conclusions

This thesis presents facile methods for synthesizing plasmonic nanostructures (NSs), specifically silver (AgNPs) and gold nanoparticles (AuNPs), through optimized processes, which could exhibit desirable optical, structural, and morphological properties. The optimized NPs were then employed for the detection of milk adulterants using three broad sensing approaches, each technique is discussed in separate chapters. The key findings of this work are presented across three chapters—Chapter II, Chapter III, and Chapter IV—and are summarized here.

The thesis begins with **first chapter** which provides a comprehensive overview of metal NPs as plasmonic NSs, emphasizing their unique properties and their applications in the development of modern-day sensing technologies. The discussion then shifts to the critical issue of milk adulteration and contamination, underscoring the limitations of existing detection methods. This chapter sets the stage for the subsequent research, highlighting the need for innovative solutions to address the shortcomings of current techniques in detecting milk adulterants.

The **second chapter** of the thesis deals with the development of colorimetric sensor for real-time sensing of milk adulterants and contaminants, which is further divided into two sections. The first section discusses substrate-free colorimetric sensing protocols developed for the detection of adulterants such as melamine, formalin, hydrogen peroxide, and salicylic acid. The second section elaborates on paper-based colorimetric sensors for detecting three adulterants (melamine, formalin, and hydrogen peroxide) and four contaminants (mercury, arsenic, cadmium, and lead).

Part A of this chapter delves into various synthesis techniques and selective functionalization strategies to optimize NPs for highly sensitive detection of individual milk adulterants. The synthesized NPs were mainly spherical in shape, exhibiting a face centred cubic (FCC) crystalline structure with an average mean diameter ranging between 8 to 18 nm. Their position of absorbance peaks aligns with the characteristic plasmonic band of AgNPs. Specifically, *Camellia sinensis*-functionalized AgNPs were developed for interference-based colorimetric sensing of melamine in milk. Antioxidants in green tea is responsible in reducing the silver ions (Ag^{+}) to AgNPs. The compound epigallocatechin gallate (EGCG) present in green tea functionalises and encapsulates the AgNPs. This functionalisation is responsible for hydrogen bonding of the surface functionalised AgNPs with melamine, resulting change in colour of the NPs solution. A limit of detection (LOD) value of 1.44 ppm was obtained with a recovery rate of 93%. Additionally, *Bombax ceiba*-functionalized AgNPs were utilized for detecting hydrogen peroxide in milk. Here, the cotton tree leaves extract was used to reduce and cap AgNPs, preventing it from aggregating. Presence of hydrogen peroxide in milk resulted in change in colour of the AgNPs to colourless. An LOD value of 8.46 ppm was obtained with a recovery rate of 92%. Further, L-cysteine-functionalized AgNPs were applied for formalin detection. Formalin causes colour change by reducing interparticle distance, leading to aggregation. This results in LOD value of 3.51 ppm and a recovery rate of 90%. Lastly, *Citrullus lanatus* rind extract-functionalized AgNPs were employed for sensing salicylic acid in milk. The watermelon rind extract was utilised both as a reducing and functionalizing agent for the selective detection. Presence of salicylic acid in milk causes a colour change from yellow to brown by reducing interparticle distance of the NPs with a LOD value of 0.55 ppm and a recovery rate of 96%.

Likewise, **Part B** of this chapter explores a novel paper-based sensing platform that uses multiple functionalized AgNPs for the simultaneous detection of various adulterants and contaminants in milk. The synthesized NPs were carefully optimized to achieve a spherical shape, with an FCC crystalline structure, and an average mean diameter ranging between 3 to 16 nm. Their absorbance peaks also align with the plasmonic band of AgNPs. Here, the fabricated platform features seven conduits. Each conduit was selectively impregnated with specific functionalized AgNPs, separated by hydrophobic barriers. The adulterated or contaminated milk yields colour changes. The specific colour change at each conduit

indicates the presence of specific adulterants. This sensing architecture is specifically designed to detect melamine, formalin, and hydrogen peroxide as adulterants, and mercury, arsenic, cadmium, and lead as contaminants. Notably, each sensing scheme achieved remarkable low detection limits, indicating high sensitivity. As for example, for detection of melamine, AgNPs were functionalized with maleic acid. Here, the anime (NH_2) groups of melamine form hydrogen bonds with maleic acid on the surface of AgNPs which results in decreased interparticle distance, causing a colour change from yellow to dark brown. For detection of formalin, AgNPs were functionalized with l-cysteine. The NH_2 groups in l-cysteine creates bond with formaldehyde, which results in stabilized interactions, and decreased interparticle distance. This leads to the colour change of AgNPs from yellow to dark brown. In similitude to formalin detection, for detection of hydrogen peroxide, AgNPs were reduced by the antioxidants present in green tea. Hydrogen peroxide oxidizes silver atoms (Ag^0) on the surface of NPs to silver ions (Ag^+). This oxidation leads to a colour change from yellow to colourless. For detection of mercury ions (Hg^{2+}), which is a milk contaminant, AgNPs were functionalized with poly vinyl alcohol (PVA) and reduced by banana root bulb (BRB) extract. The Hg^{2+} ions interact with hydroxyl groups in PVA and gets reduced to Hg^0 converting the NPs back to ionic form. This oxidative dissolution of AgNPs results in a colour change from yellow to colourless. For detection of arsenic ions (As^{3+}), AgNPs were functionalized with citric acid. Here, the As^{3+} bind to carboxyl groups of citric acid. Cross-linking and aggregation of NPs, causes a colour change from yellow to black. For detection of lead ions (Pb^{2+}), AgNPs were functionalized with l-glutamine. The Pb^{2+} form coordination complexes with amine and carboxyl groups of l-glutamine which results in aggregation of NPs. This leads to a colour change from yellow to dark brown. For detection of cadmium ions (Cd^{2+}), AgNPs were functionalized with salicylic acid. Here, the Cd^{2+} bind to hydroxyl and carboxyl groups of salicylic acid which leads to aggregation of NPs, causes a colour change from yellow to dark brown.

The **third chapter** explores localised surface plasmon-based sensing systems for the detection of milk adulterants, specifically melamine and hydrogen peroxide, with AgNPs selectively functionalized for each target adulterant. Maleic acid-functionalized AgNPs were employed for melamine detection by immobilizing these AgNPs on a glass substrate, which was then placed in a cuvette for analysis using UV-Vis spectroscopy. The

synthesized NPs were meticulously optimized to exhibit a characteristic absorbance peak at 407 nm, an FCC crystalline structure, and an average diameter of 3.90 nm. In presence of melamine, the sensor containing the immobilised NPs displayed a notable absorbance peak shift, caused by the interaction between the immobilized NPs and melamine. This was used to formulate a calibration curve and estimate the LOD which was found to be 10.48 ppb with a recovery rate of 96%-99.4%. Similarly, Tulsi leaves extract-reduced and PVA functionalized AgNPs were utilized for detecting hydrogen peroxide in milk. In similitude, the synthesised AgNPs displayed a characteristic absorbance peak at 434 nm with an FCC crystalline structure and mean diameter of 19.47 nm. Similarly, a notable peak shift was obtained as a result of interaction between the immobilized AgNPs and hydrogen peroxide. This was used to plot a calibration curve and estimate the LOD which was found to be 2.72 ppb with a recovery rate between 97%-108%.

Meanwhile, **chapter four** delves into various electrochemical approaches for the detection of milk adulterants. For urea detection, (3-Aminopropyl) triethoxysilane (APTES)-coated, green tea-reduced AuNPs were deposited onto indium tin oxide (ITO) coated glass slides, serving as the working electrode in cyclic voltammetry. Similarly, melamine detection employed polyethylene Glycol (PEG)-coated, maleic acid-functionalized AgNPs on ITO electrodes, while hydrogen peroxide detection utilized polyvinylpyrrolidone (PVP)-functionalized AgNPs. The NPs synthesized for these sensors were meticulously optimized to align their absorbance bands with plasmonic bands of the NPs. These NPs, with sizes ranging between 10–20 nm, exhibited an FCC crystalline structure. Calibration plots were constructed for each sensor to determine the LOD and sensitivity, confirming the effectiveness of the electrochemical sensing strategies. The NP-based sensors demonstrated the capability to detect trace levels of adulterants in milk. For urea detection, APTES-functionalized, green tea-reduced AuNPs on ITO-coated glass slides displayed a concentration-dependent increase in peak current, achieving a LOD of 4.02 ppm and a recovery rate between 95.05%- 99.44%. Melamine detection was achieved with PEG-coated, maleic acid-functionalized AgNPs on ITO slides, exhibiting a clear oxidation peak current increase with concentration, resulting in a LOD of 12.24 ppb and a recovery rate between 97.73%- 101.72%. Finally, hydrogen peroxide detection was performed using PVP-capped, biologically reduced AgNPs on ITO slides, which showed a distinct reduction peak current corresponding to hydrogen peroxide concentration, with

a LOD of 5.19 ppb and a recovery rate 98.73%- 100.45%. These results underscore the potential of plasmonic NPs-based electrochemical sensors as robust, sensitive, reproducible, and selective tools for detecting adulterants in milk, achieving low LOD and high recovery rates across multiple analytes.

Finally, the last chapter, **chapter five** presents a comprehensive comparative study, where the executed works in this thesis is meticulously compared with previously reported studies in the literature. This analysis highlights the innovative aspects and novelty of the present research. Additionally, a comparative evaluation of all sensing architectures introduced in this work is provided, emphasizing the advancements and contributions made by the current study.

The conclusive summary of all the executed works in this thesis is presented in **Table 6.1**.

6.2 Future prospects

Milk adulteration is a critical issue, with cases of food poisoning caused by the consumption of adulterated milk increasing steadily. To address this challenge, the development of innovative, next-generation sensors is being actively explored. While this thesis highlights fabrication of several point of care sensors, still some approaches remain unexplored for detecting these adulterants. The current research can be further extended and potentially applied across various domains. Some promising future directions are outlined below.

1. ***Implementation of SERS-Based Scheme:*** Surface-Enhanced Raman Spectroscopy (SERS) offers a highly sensitive technique for detecting trace levels of contaminants, making it a promising tool for milk adulteration detection. SERS based system amplifies Raman signals through the interaction of molecules with metal NSs. This amplification can enable the detection of even minute quantities of adulterants that may go undetected by conventional methods. SERS could be used to identify adulterants like melamine, urea, or hydrogen peroxide with high precision, providing rapid and reliable results. Its ability to detect low-concentration contaminants makes it invaluable for ensuring safety and quality of milk.

2. ***Development of Nanocomposite Sensors:*** Nanocomposite sensors combine multiple functional materials, such as NPs, polymers, and carbon-based materials, into a single sensing platform. This approach exploits the unique properties of each component thereby enhancing the sensitivity, selectivity, and overall performance of the sensor. For instance, incorporating conductive nanomaterials (e.g., graphene, carbon nanotubes) can improve the sensor's electrical conductivity, whereas metal NPs tunes plasmonic properties for signal amplification. These hybrid materials can be tailored to detect specific milk adulterants more effectively than traditional sensors. Moreover, nanocomposites can be engineered to offer resistance to interference from the complex matrix of milk, thus improving accuracy. Moreover, the fabrication of advanced nanocomposite sensors will significantly enhance the sensitivity and specificity of milk adulterant detection, enabling the identification of a broader range of contaminants with higher accuracy.

3. ***Multiplexed Detection Platforms:*** The development of multiplexed sensors is a key advancement for milk adulteration detection. These platforms allow the simultaneous detection of multiple adulterants or contaminants in a single test, improving efficiency and reducing testing time. A multiplexed platform could integrate several detection techniques (e.g., electrochemical, plasmonic, colorimetric) into one system, allowing the identification of various adulterants such as melamine, formalin, hydrogen peroxide, and heavy metals in a single sample. This would be especially useful for large-scale testing of milk and its products, especially in milk processing plants, where rapid and comprehensive quality assessments are needed. Moreover, they also allow real-time analysis of multiple adulterants and contaminants in one streamlined process, saving both time and resources.

4. ***Portable and User-Friendly Devices:*** The future of milk adulteration detection lies in the development of easy-to-use, portable devices that can be employed directly in the field. Such sensors would help in real-time assessment of milk quality at various stages of the supply chain. These portable sensors can be designed in such a way so that they can also be used by non-experts, requiring minimal pre-processing step. Portable devices for adulterants detection can incorporate sophisticated technologies like electrochemical sensors, plasmonic, or SERS into compact, handheld units, enabling on-site detection of adulterants without the need for laboratory equipment. A portable,

user-friendly detection devices will help in ensuring continuous milk quality monitoring throughout the supply chain and reducing the risk of adulteration.

Table 6.1: *Summary of all the detector parameters of all the sensing architectures.*

Sensing Unit	Material	Adulterant / Contaminant	LOD	Sensitivity (Change in absorbance or absorbance ratio /ppm or ppb)	Recovery
Colorimetric (without substrate)	GT-AgNPs	Melamine	1.44 ppm	0.01	93%
	CT-AgNPs	Hydrogen Peroxide	8.46 ppm	0.03	92%
	L-cyst-AgNPs	Formalin	3.51 ppm	0.002	90%
	WR-AgNPs	Salicylic Acid	0.55 ppm	0.06	96%
Colorimetric (Paper based)	MA-AgNPs	Melamine	0.76 ppm	-	-
	AgNPs	Hydrogen Peroxide	5.60 ppm	-	-
	L-cyst-AgNPs	Formalin	4.56 ppm	-	-
	PVA capped BRB reduced AgNPs	Mercury	0.87 ppm	-	-
	CA-AgNPs	Arsenic	0.65 ppm	-	-
	L-glu-AgNPs	Lead	0.35 ppm	-	-
	SA-AgNPs	Cadmium	0.73 ppm	-	-
LSPR based System	BSA/Maleic acid/AgNPs/APTES	Melamine	10.48 ppb	0.11	96% - 99.4%
	BSA/PVA/OT-AgNPs/APTES	Hydrogen Peroxide	2.72 ppb	0.46	97% - 108%
Electrochemical System	APTES/GT-AuNPs/APTES/ITO electrode	Urea	4.02 ppm	7.45×10^{-4} mA/ppm	95.05% - 99.44%
	PEG/MA-AgNPs/APTES/ITO electrode	Melamine	12.24 ppb	2.45×10^{-3} mA/ppb	97.73% - 101.72%
	PVP/AgNPs/APTES/ITO electrode	Hydrogen Peroxide	5.19 ppb	6.35×10^{-3} mA/ppb	98.73% - 100.45%