ABSTRACT

Developing Facile Plasmonic Nanostructures and their Application in Sensing Prominent Adulterants in Cattle Milk

Nanomaterials (NMs) exhibit exceptional properties compared to their larger counterparts. This is strongly influenced by their morphological and structural characteristics. These materials are classified based on their dimensions in the nanoscale regime, with nanoparticles (NPs) being zero-dimensional (0D) structure. Among various NPs, plasmonic NPs commonly composed of noble metal NPs have gathered significant attention due to their wide range of applications especially in the field of sensing.

Metal NPs contain abundant free conduction electrons that oscillate when exposed to light of wavelength comparable to the size of the NPs. At resonance, these electron oscillations match the frequency of the external electromagnetic radiation, resulting in a phenomenon termed as localized surface plasmon resonance (LSPR), which significantly enhances the light absorption by the NPs. By altering the structure, morphology, composition, or external environment of plasmonic nanostructures (NSs), the resonance wavelength can be tuned. These parameters of the NPs can be altered by optimising the process of synthesis of the NMs.

The optical characteristics of these NPs can be adjusted through unique functionalization routes. Due to their pronounced absorbance at resonance, they serve as effective colorimetric and plasmonic sensors. Any inherent change in the plasmonic behaviour of metal NPs is caused by a visible alteration in their colour, which is due to changes in interparticle distance upon exposure to specific analytes or may be due to the conversion of metallic ions from NPs when interacting with analytes possessing strong oxidizing properties. Additionally, these NMs also function as plasmonic sensors by detecting variations in the refractive index of the surrounding medium induced by binding of an analyte with the functionalized NPs, which thereby triggers a significant change in the position of the absorbance peak. Apart from this, plasmonic NPs are also employed as electrochemical sensors due to their high surface area-to-volume ratio, which enables significant analyte adsorption on the surface of functionalised NPs. They offer high

selectivity, rapid response times, reusability and stability as they remain intact even after multiple cycles.

Milk adulteration is a significant global issue that has been persisted over centuries. It is amongst the top 10 most adulterated food items worldwide. Adulteration involves the addition of undesirable foreign substances to milk, which degrades its quality and makes it unsafe for consumption. Various adulterants are added to milk to increase the non-protein nitrogen content by introducing toxic nitrogen-rich compounds like melamine and urea. Additionally, some toxic and carcinogenic adulterants such as hydrogen peroxide, formalin and salicylic acid are added to extend milk's shelf life, which prevents its spoilage due to growth of microorganisms. Regular consumption of these adulterants can cause serious health hazards such as gastrointestinal problems, kidney stones, cancer, food poisoning, and severe infections. To prevent such instances, regulatory bodies across the globe have set maximum permissible limits in parts per million (ppm) and parts per billion (ppb) on the presence of these adulterants in milk and other food items: for melamine (2.5 ppm); for urea, (700 ppm); for hydrogen peroxide, (5 ppm); and for formalin, (4 ppm).

Additionally, milk contamination by heavy metal (HM) ion residues is a significant issue of this decade. Various types of HM ions, primarily mercury (Hg), arsenic (As), cadmium (Cd), and lead (Pb), are present in milk. These contaminants often get introduced during the pasteurization process. The consumption of these metals is equally dangerous, and regulatory bodies have also set maximum permissible limits for them as well: for Hg, (2 ppb); for As, (10 ppb); for Cd, (5 ppb); and for Pb, (10 ppb).

Given the aforementioned facts, it is crucial to properly diagnose and quantify these hazardous adulterants and contaminants. Currently, the sensing procedures for detecting these substances are highly sophisticated and thus, require trained personnel, which hinders regular monitoring of milk quality. Therefore, there is an urgent need to develop point of care user-friendly detection techniques capable of identifying harmful adulterants and contaminants just above permissible limits.

In response to this concern, our research focuses on the streamlined one-pot synthesis of plasmonic NSs using environmentally friendly or chemical pathways, coupled with appropriate surface functionalization. Precise functionalization of NPs results in lower limits of detection (LOD) and enhanced recovery rates. This is achieved by selective binding functionalized NPs with the targeted analyte, causing significant changes in the plasmonic profile of the NPs. This plasmonic shift enables the qualitative and quantitative assessment of adulterant concentration in milk samples via a cost-effective route.

For detection, we have primarily chosen three routes: colorimetric sensing, localized surface plasmon-based sensing, and electrochemical sensing. The detection of various adulterants through these routes is presented in the thesis in the form of various chapters. The outcomes of the entire study are organized into several parts in the form of chapters. The thesis is arranged as follows.

Chapter I begins by introducing the various categories of metal NPs as plasmonic NSs and their utilization in detection of toxic milk adulterants. The chapter then provides a general introduction on the problem milk adulteration and contamination. Then the discussion shifts towards plasmonic NPs, their properties and synthesis routes. Further, the use of these NPs is discussed with a particular focus on their sensing applications towards detection of milk adulterants, highlighting the inadequacies of previously reported sensing techniques for the detection of these adulterants. Finally, the chapter concludes with the objectives and outline of the thesis.

The ***Chapter II*** is subdivided into two subsections. Where the ***first part*** focuses on the utilisation of selective functionalized of NPs toward colorimetric detection of milk adulterants without the use of any substrate. *Camellia sinensis* leaves-functionalized silver nanoparticles (AgNPs) were used for interference-based colorimetric sensing of melamine in milk. *Bombax ceiba* leaves-functionalized AgNPs were employed for sensing hydrogen peroxide in milk. L-cysteine-functionalized AgNPs were used for sensing formalin in milk. Similarly, *Citrullus lanatus* rind-functionalized AgNPs were utilized for sensing salicylic acid in milk. The UV-Visible (UV-Vis) response of these functionalized NPs after interaction with the targeted adulterant was used to obtain a linear calibration plot, which was utilized to determine the selectivity, sensitivity, recovery, and LOD.

In the ***second part*** of ***Chapter II***, a unique method is discussed where various functionalized AgNPs were used to selectively detect multiple adulterants and contaminants simultaneously via a paper-based sensing scheme. The paper contains seven conduits, with each conduit end selectively impregnated with functionalized NPs and separated by a hydrophobic barrier. When adulterated and contaminated milk is poured at the centre of the substrate, it flows through all the seven channels. The colour change at the end of the conduits indicates the presence of those specific adulterants or contaminant.

Further, Red, Green, & Blue (RGB) and Hue, Saturation, & Value (HSV) data obtained from the colorimetric change on the paper-based platform were utilised to determine the calibration plots, which were then used to calculate the LOD for each adulterant and contaminant. This sensing architecture specifically targets the detection of melamine, formalin, and hydrogen peroxide as adulterants, and Hg, As, Cd, and Pb ions as contaminants. The LOD obtained for each sensing scheme was quite low, with high sensitivity.

Chapter III reports localized surface plasmon-based sensing of various adulterants. NPs were selectively functionalized for the detection of each adulterant. Firstly, maleic acid-functionalized AgNPs were used for sensing melamine. To achieve this, the NPs functionalised with maleic acid were immobilized on the surface of a glass substrate. This glass chip, containing immobilized functionalized NPs, was used for detection by placing it in a cuvette cell and using a standard UV-Vis spectrophotometer to obtain the results. A significant peak shift was observed due to the interaction of the immobilized NPs with melamine, which was used to obtain a calibration plot, and to estimate the LOD for the milk adulterant. Similarly, polyvinyl alcohol (PVA) coated-*Ocimum tenuiflorum* leaves reduced AgNPs were used for detecting hydrogen peroxide. Similar peak shifts were observed, which were calibrated to calculate the LOD for the presence of hydrogen peroxide in milk.

Chapter IV discusses various electrochemical schemes for detecting different adulterants. For detecting urea in milk, (3-Aminopropyl)-triethoxysilane (APTES) coated *Camellia sinensis* leaves-reduced gold nanoparticles (AuNPs) coated onto an indium tin oxide (ITO) electrode were utilized. The coated ITO electrode served as the working electrode, where cyclic voltammetry (CV) cycles were run, and the oxidation peaks obtained were analysed. With an increase in urea concentration, the oxidation peak increased, indicating presence of urea in milk. Similarly, melamine was detected electrochemically using polyethylene glycol (PEG)-coated, maleic acid-functionalized-AgNPs deposited on ITO coated glass as the working electrode. Here, CV cycles were run, and the oxidation peaks were studied, which increased with the rising concentration of melamine in milk. Finally, hydrogen peroxide was sensed electrochemically using polyvinyl pyrrolidone (PVP)-functionalized AgNPs coated ITO glass slide as the working electrode, where instead of oxidation, the reduction peaks was obtained which got intensified with increase in hydrogen peroxide concentration in milk. Finally, a linear calibration graph was obtained to determine the LOD and sensitivity for each adulterant.

Chapter V, presents a comprehensive comparative study, where the current work 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.

Chapter VI presents a summary of all the reported works and important findings of the research. It also explores prospects for future investigation of the presented work.