Review Article
DOI: https://www.doi.org/10.62418/ijvph.11.1.2025.7-15

Quantum Dots in Milk Adulteration

Preetam Banerjee[(1)], Pramit Chakraborty[(1)], Anindita Debnath[(2)], Chaitali Chakraborty[(3)], Pradip Kumar Roy[(4)], Adarsh M. Kalla[(5)], Subhadip Manik[(6)], Partha Pratim Debnath*[(2)]

[(1)]PG scholar, Department of Dairy Science and Food Technology, Institute of Agricultural Sciences, Banaras Hindu University,Varanasi-221005, Uttar Pradesh; [(2)]Assistant Professor, [(3)]Associate Professor, [(4)]Professor, Faculty of Dairy Technology, West Bengal University of Animal and Fishery Sciences (WBUAFS), Mohanpur-741252, Nadia, West Bengal; [(5)]Assistant Professor, Department of Dairy Engineering, Dairy Science College, Mahagaon cross, Kalaburagi-585316, Karnataka; [(6)]PhD Scholar, Department of Dairy Technology, National Dairy Research Institute (NDRI), Karnal, Haryana

(Received: 23rd April 2025 | Accepted: 15th June 2025)

Abstract

Adulteration of milk and milk products is a serious concern for the dairy industry. Adulteration not only cheats the consumer, but also results in various health implications. Various methodologies have been devised over decades for detecting adulterants in milk. However, some of these methods have limitations in sensitivity, while others; although very sophisticated and sensitive, involve complicated sample preparation. Quantum dots with versatile applications have shown competency in overcoming the above said limitations. Quantum dots exhibit excellent fluorescent characteristics, which form the basic foundation for their usage in this field. In this paper, we discuss the different types of quantum dots which have been used over the recent years to detect various adulterants which are commonly found in milk.

Keywords: Quantum dots, Milk, Adulteration

Introduction:

Quantum Dots (QDs) are semi-conductor non-crystals that are typically composed of group II-VI or group III-V elements such as Cadmium Selenide or Indium Phosphide. Mostly, these are coated with a shell material like zinc sulfide to enhance their stability and quantum yield (Bonilla et al., 2016). Size of QDs basically ranges between 1 to 10 nm. The fluorescence properties of QDs make them ideally suitable for various set of applications. These fluorescence characteristics are primarily because of the quantum confinement effects of QDs. In simple words, due to quantum confinement, the size of particle decreases, which leads to increase in energy bandgap. This alteration in energy bandgap results in emission of light rays of shorter-wavelength (higher energy) (Xiao-Yue et al., 2020). Although the phenomenon of the fluorescence emergence of QDs is debatable, the fluorescence property of QDs relies on electron excitation and relaxation processes. Other reported mechanisms comprise of the quantum size effect, surface defect states, and molecular and molecule like states. In nutshell, when a specific wavelength of a photon is absorbed by a quantum dot, an electron is excited to a higher energy state, leaving behind a hole with a positive charge. Upon recombination of electron and hole, fluorescence is released in the form of energy. This fluorescence depends highly on the quantum dot size and composition. Accordingly, the main mechanisms that contribute to the detection capabilities of the QDs can be categorized into FRET (Fluorescence resonance energy transfer), PET (Photo-induced electron transfer), fluorescence quenching and IFE (Internal filter effect).These properties help QDs to be employed in bio-sensors, bio-imaging, and detection systems of chemicals and contaminants, including identification of adulterants in milk (Shi et al., 2019).

In dairy industry, QDs have gained attention for their potential usage in detecting adulterants and contaminants in milk. Their fluorescence-based detection capabilities result in high sensitivity in identifying adulterants even at trace levels. In contrast to other advanced adulteration detecting methodologies, such as chromatography, spectroscopy and immunoassays, QDs provide rapid results. This makes the process of detection of milk adulterants more efficient and cost-effective. Milk adulteration refers to the intentional addition of foreign substances, either to increase volume, enhance appearance, or falsely boost fat or protein content, that ultimately leads to the economic benefit of the producers. Adulterants can be broadly categorized into non-toxic adulterants (e.g. water, starch, skim milk powder and many others) and toxic adulterants (e.g. melamine, detergents, formalin, hydrogen peroxide and urea). While dilution with water is a common form of adulteration that lowers nutritional value, toxic adulterants are particularly dangerous and can lead to severe health complications such as gastrointestinal disorders, kidney failure, and cancer (Poonia et al., 2016).

Given the severity of these adulterants, rapid and highly sensitive detection devices are required to ensure food safety and public health. A considerable number of

adulterant identification methodologies are available and are quite prevalent, but because of their limitations in sensitivity and reproducibility, the shift is towards advanced adulteration detecting methodologies which can overcome the aforesaid limitations. Some of them are High-Performance liquid Chromatography (HPLC), Gas chromatography-mass spectrometry (GC-MS), and near-infrared (NIR) spectroscopy. However, these techniques often require complex sample preparation, skilled personnel, and high-cost instruments. Time required for the detection of the samples play an important role as well. Quantum Dot-based technologies offer a promising alternative due to their high sensitivity, real-time detection capability and ease of use (Nagraik et al*.,* 2021).

Quantum dot-based sensors offer a rapid and highly sensitive alternative for detecting milk adulterants. The functionalization of QDs with selective recognition molecules, such as antibodies or aptamers, enables the detection of specific adulterants through fluorescence quenching or enhancement mechanisms (Shalileh et al*.,* 2023). Recent applications of QDs in milk adulterant identification include fluorescence-based detection, bio-sensors and establishment of better forms of E-nose and E-tongue. In fluorescence-based detection, QDs can be functionalized to bind specific adulterants, producing a fluorescence signal upon interaction. This approach has been used to detect melamine, urea and formaldehyde in milk samples. QD -based biosensors have been developed to detect enzymatic changes caused by adulterants. QDs have also been incorporated into electronic nose and tongue systems for real-time adulterant detection, thereby improving the accuracy and efficiency of milk quality assessment (Srivastava et al., 2017). QDs have been widely employed to detect heavy metals, food borne pathogens, food additives, pesticide residues, veterinary drugs, adulterants, nutritional components and many others (Xiao-Yue et al*.,* 2020). In this paper, we keep our focus restricted to the application of QDs in milk adulteration only.

Methodologies for detecting adulteration in milk:

Milk adulteration remains a major public health concern. Traditional physico-chemical methods for inspecting adulterants comprise pH, density, alcohol, clot-on-boiling, freezing point assessments and many others. The lactometer test measures the specific gravity or density of milk. Adulteration with water reduces the density, while thickening agent's incorporation increases it. (Poonia et al., 2016). Alcohol and clot-on-boiling test detects presence of detergents, starch and other contaminants. Presence of synthetic chemicals indicates a positive COB test which refers to protein denaturation (Bonilla et al*.,* 2016). Water adulteration can also be detected by measuring the depression in freezing point. Unadulterated milk freezes at -0.54°C; any deviation suggests dilution with water (Xiao-Yue et al., 2020). Although the aforesaid methodologies provide a cost-effective approach, but are sometimes imprecise for detecting few adulterants such as water, urea, starch and so forth. As a result, there has been a paradigm shift to advanced adulterant detecting techniques, which is being discussed as follows.

Recent cutting edge adulteration technologies include chromatographic techniques, such as HPLC and GC-MS. These techniques enable precise quantification of adulterants like urea, melamine, formaldehyde and many others, but require expensive equipment and skilled personnel (Bonilla et al*.,* 2016). In addition, these methodologies are quite time consuming and the sample preparation is quite complex. Apart from the aforesaid, industries accepted the application of FTIR (Fourier infrared spectroscopy) and Raman spectroscopy as rapid adulteration detection techniques. FTIR is a nondestructive technique based on their unique spectral signatures (Xiao-Yue et al., 2020). Raman spectroscopy provides molecular fingerprints of milk components and is being utilized in detection of synthetic adulterants (Poonia et al*.,* 2016). In recent years, biosensor-based adulterant detection methods have also gained popularity. Few of these sensors, includes surface plasmon resonance (SPR), electrochemical, enzymatic and immuno bio-sensors. Enzymatic Biosensors detects urea, hydrogen peroxide and other adulterants based on enzyme-substrate interactions, subsequently producing measurable electrochemical signals (Nagraik et al*.,* 2021). Immunosensors are antibody-based biosensors which provide high specificity for contaminants such as antibiotics and pesticides (Shalileh et al*.,* 2023). Although bio-sensors offer portability and high specificity, but it requires further validation for routine uses. Meanwhile, immunological techniques such as Enzyme-Linked Immunosorbent Assay (ELISA) offer high specificity for detecting protein-based adulterants but demand specialized antibodies and laboratory settings. Furthermore, Polymerase Chain Reaction (PCR) provides molecular-level detection of adulterants, particularly for identifying milk from different species, though it is ineffective for non-DNA-based adulterants (Deng et al., 2020). Other emerging techniques, such as electronic noses and tongues, analyze volatile compounds and taste patterns to detect adulteration and spoilage, though they require extensive calibration to improve specificity (Tudor Kalit et al., 2014). These methodologies, when integrated with chemometric tools, promise more robust and accessible detection frameworks for ensuring milk quality, thus marking it as safe. The following table (Table 1) summarizes various adulterant detecting techniques, underlying principle and limitations.

Table 1: List of methods for detection of adulterants

S. No	Methods	Principle	Target Adulterants	Limitations	References
1.	Physico-chemical methods	Change in pH, density, colour and refractive index	Water, urea, starch, detergents	Less precise, labor-intensive, requires multiple tests	Kamthania et al., 2014
2.	Spectroscopy (FTIR, Raman)	Detection of molecular vibrations and interactions	Urea, melamine, formaldehyde, whey proteins	Expensive, **requires** complex data analysis	Xiao-Yue et al., 2020
3.	Chromatography (HPLC, GC-MS and many others)	Separation and detection of compounds based on chemical properties	Melamine, formalin, whey proteins, nitrogenous compounds	Expensive equipment, time-consuming	Joolaei Ahranjani et al., 2025
4.	Immunological techniques	Antigen-antibody interaction for specific detection	Melamine, whey proteins, exogenous proteins	Requires specific antibodies, labour-intensive	Chi et al., 2024
5.	Bio-sensors (SPR, Piezoelectric and optical Amperometric)	Biological recognition elements detect analytes via signal transduction	Urea, melamine, formalin, proteins	Requires validation, expensive sensors	Nagraik et al., 2021
6.	PCR	DNA-based identification of adulterants	Foreign milk species, microbial contamination	Cannot detect non-DNA adulterants	Deng et al., 2020
7.	Electro-migration(SDS-PAGE, Capillary Electrophoresis, Isoelectric Focusing)	Separation of proteins based on molecular weight or charge	Whey proteins, exogenous proteins	Low reproducibility, complex analysis	Acunha et al., 2016
8.	Electronic nose and tongue	Detects volatile compounds and taste patterns	Synthetic milk, detergents, spoilage indicators	Lacks specificity, requires calibration	Tudor Kalit et al., 2014

Concept and Mechanism of Quantum Dots (QDs) in milk adulteration:

Quantum dots are versatile accessories, which can be used in multiple areas in food and dairy product analysis. QDs have specific nanoscale properties, comprising of large surface area, heightened surface reactivity, quantum confinements and many others. Some of these properties can be enhanced and can be modified so as to make it suitable as an adulterant detecting tool. For instance, fluorescent activity, light scattering and electrochemical properties of QDs can be boosted by modifying its size resulting in accurate detection of adulterants in milk (Valdes et al., 2009; Perez-Lopez et al., 2011)

Various adulterants in milk can be detected instantly when QDs forms bonds with affinity ligands and antibodies. There are certain quantum dots like carbon dots (CQDs) that shows various intensities of fluorescence depending on the pH. Milk has a pH of 6.7, but, on microbial spoilage its pH reduces, while on addition of adulterants like urea, melamine or other nitrogenous source, an increase in pH is observed. This change in pH can be easily detected if milk is being inspected with CQDs (Pang, 2020). A study was carried out by Choudhary and associates to co-relate CQD's fluorescence properties with pH, in the presence of an adulterant. The authors reported that with increase in pH, carboxyl groups present in the CQDs surface ionize, leading to increased deprotonation at higher pH, which enhances fluorescence intensity (Choudhary et al*.,* 2021).

The suitability of QDs as an adulterant inspecting tool is because of its fluorescence activity under short range UV light, low cost, ease of production, biocompatibility and low toxicity. Here, we are going to summarize various researches, which have been carried out in this field by using QDs in various forms.

Application of QDs in milk adulteration:

Adulteration with Urea

Milk is a perishable commodity. Urea is used as a preservative to increase the shelf life of milk (Huang et

al., 2007). Yin and his scientific group applied a one-step manufacturing approach to produce stable green-light-emitting carbon dots (CQDs) as a fluorescent probe. When exposed to light with a wave length between 350 and 450 nm, these CQDs became fluorescent intensely. However, a gradual decrease in fluorescence intensity was observed with an increase in urea level, which made them suitable as a sensitive urea detection tool. The limit of detection (LOD) for urea in this setup was 6.27 mg/L, thereby proving the efficacy of these CQDs for precise and efficient urea sensing (Yin et al*.,* 2021).

Similarly, Alanazi et al. (2024) developed a ratiometric fluorescence sensor for urea detection using carbon dots (CQDs) with pH-responsive quenchers. Here, red-emitting CQDs (R-CQDs)/methyl red (MR) and near-infrared emitting CQDs (NIR-CQDs)/Cu^{2+} were utilized for urea detection.The change in fluorescence intensity of these CQDs is based upon pH alteration. This change in pH is because of urease enzyme which hydrolyses urea to create ammonia. As a result, pH gets elevated and fluorescence intensity changes. At neutral pH, MR's red colour blocks R-CQD's emission; however, when pH rises, MR becomes yellow, reviving R-CQD's fluorescence. At increasing pH levels, Cu^{2+} promotes NIR-CQD quenching, which reduces fluorescence. Nevertheless, the urea level is ascertained by monitoring the fluorescence ratio between R-CQDs and NIR-CQDs. The developed sensor was capable to detect urea in very minute quantities, even as low as 0.00028 mM. In addition, high recovery rate of CQDs was reported in levels of about 96.5% to 101.0%.

In another study, Li et al. (2021) developed Fe/N-co-doped carbon dots for detection of urea. The developed sensor was capable to detect urea even in very minute concentration, for instance, 0.001 µM.

Adulteration with Formaldehyde

Formalin or formaldehyde is also utilized as a preservative in milk. Usage of the said component in milk can cause serious health concern. Graphene Quantum Dots (GQDs), a type of CQD, has strong luminescence and are optically stable and can be used to detect formaldehyde (Zhao et al., 2015). However, traditional CQD-based probes are limited to aqueous samples and require long interaction times. To overcome this, Headspace Single-Drop Micro-Extraction (HS-SDME) with GQDs has been developed which enables efficient formaldehyde detection. For this purpose, silver Modified Nitrogen Doped GQD (N-GQDs-Ag) was made which acts as a selective photoluminescent probe (Padilha et al., 2022). This probe can be synthesized via a hydrothermal method using citric acid, urea and Tollen's reagent. The probe developed absorbs formaldehyde vapour when suspended above the milk sample at the headspace. Rapid, high-sensitivity detection with minimal sample preparation is possible in this method. The prepared N-GQDs-Ag dispersion emits visible blue colour luminescence when exposed under UV radiation, which is due to oxidation of GQDs as it is exposed to atmospheric O_2. Usage of silver in this dispersion, enhanced the photoluminescence intensity by four times and also facilitated in retaining half of its original photoluminescence intensity for 60 days as opposed to 20 days for N-GQDs alone. For the detection of milk adulterants, this stability guarantees constant and trustworthy indications. Silver was protected even at high pH levels because N-GQDs-Ag inhibited Ag oxidation. On the other hand, photoluminescence dramatically dropped at pH values that were acidic (3.0–4.0). Tests revealed that although other aldehydes require extremely high concentrations to elicit quenching, however, volatile organic compounds (VOCs) from the ketone, alcohol, ethyl ester, nitrogen-containing, and dimethyl sulfone classes had no impact (≈1% quenching). This validated the volatile formaldehyde selectivity.

Similarly, another research has been carried out by Mostafapour et al. (2021), where Molybdenum disulfide quantum dots have been developed for detection of formaldehyde in cow milk.

Adulteration with Melamine

Melamine can increase the protein content of milk, however, usage of it have serious health implications. QDs have also been used to identify melamine adulteration in milk by combining gold nanoparticles (AuNPs) with carbon dots (CQDs) (Dai et al*.,* 2014). The amino group in melamine binds to AuNPs, thereby lowering the FRET (Fluorescence resonance energy transfer) effect and increases fluorescence intensity. As the amount of melamine grows, carbon dots show more fluorescence. The great sensitivity of this approach, allows it to detect melamine at concentrations as low as 36 nM, which makes it a perfect tool for food safety. It exhibited very high accuracy (recovery 90.47%–111.35%) and low RSD (Relative Standard Deviation) of 2.05% (Dai et al*.,* 2014). In another study, Fathima Anjila et al. (2024) developed Tb-doped GQDs that acts as a fluorescent probe. Here, the maximum detection limit was 0.31 µM and the probe was capable to detect and quantify the concentration of melamine even at minute levels of 0-5 µM. Another study reported the application of Au carbon quantum dots nano-composites for detection of melamine. Here, fluorescence and smartphone were the mode of detection, with level of detection as low as 4×10^{-3} µM (Hu et al., 2019). According to Üzek et al. (2021), the detection limit was significantly enhanced to 1.11 µM by depositing cadmium sulphide quantum dots on a molecularly imprinted shell using mini-emulsion polymerization. This resulted in specific recognition sites

on the nano-composite's surface for melamine detection. Xue et al. (2020) prepared FRET biosensor, based on protoporphyrin IX and GQDs for melamine detection. This bio-sensor was capable to detect melamine at levels of 3.6×10^{-3} µM in milk samples.

Li and co-workers also developed a quick fluorescent detection method for melamine based on charge transfer quenching of the fluorescence for GQDs in the presence of Hg^{2+}. Melamine increases the concentration of Hg^{2+} on the surface of GQDs and decreases their fluorescence by reacting with mercury through the nitrogen atoms in its amine and triazine groups. Melamine serves as the connecting agent and the charge transfer from the GQDs to Hg^{2+}causes the quenching (Li et al., 2014). Again, Ma et al. (2013) produced Cyclodextrin-decorated silver nanoparticles (CD-AgNPs) for melamine detection, using SERS (Surface enhanced Raman Spectroscopy) as the mode of detection. The CD-AgNPs detected melamine even in contents of 2.37×10^{4}µM. Likewise, Demirhan et al. (2015) developed a sensitive and simple method for detecting melamine in dairy products by using l-cysteine-capped Mn-doped zinc sulfide (ZnS) quantum dots as a probe. With a detection limit of 5.95 ng/mL in 10 mM phosphate buffer (pH 7.4), it was demonstrated that melamine quenches the probe's phosphorescence intensity. The phosphorescence intensity of Mn-doped ZnS QDs fluctuated with pH, as a result, it was inferred that, the ideal pH for detection was 7.4. The probe's signal was unaffected by the presence of ions including Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}, even at 500-fold concentrations. With this probe, melamine in dairy products may be precisely identified without any interference (Demirhan et al., 2015). In another research study, Murugesan et al. (2023), developed silver conjugated orange peel waste derived carbon dots for melamine detection. Melamine in milk interferes with the FRET between Ag particles and CQDs, thereby increasing the fluorescence intensity of CQDs.

Adulteration of goat/ewe milk with cow milk

There are many persons who suffer from cow milk protein allergy. These persons basically look for alternatives of cow milk such as goat milk, sheep milk and many others. As a consequence, adulteration of goat's milk with cow's milk is prevalent, because of manufacturer's intention to earn higher profit (de la Fuente et al., 2005; Azad et al., 2016).

Using QD tags, Kokkinos et al. (2016) developed a duplex electrochemical immunoassay to detect goat milk that has been adulterated with cow milk. The device measures both bovine immunoglobulin G (bIgG) and bovine casein (CN). The CN assay identified adulteration below 1% (v/v), but the bIgG assay detected it up to 50% (v/v) without dilution. Another study was carried out for electrochemical detection of cow's milk in ewe/goat's cheese by Livas et al. (2021). The group created a disposable antimony/tin nano-composite immunosensor in a 3D-printed microcell. The setup was able to catch even 0.07% (v/v) cow milk in the non-bovine cheese.

Adulteration with Hydrogen Peroxide

Hydrogen peroxide (H_2O_2) is a compound which possesses anti-microbial effect, because of which it is being used as a sterilizing agent of packaging materials. But, its direct addition in milk as a preservative is restricted due to its toxic nature. As a result, it is banned in several countries. Adulteration of milk by H_2O_2 is quite prevalent because of its shelf life enhancement property through microbial inhibition (Juven et al., 1996). H_2O_2 incorporation results in destruction of vitamin A and E in milk and is harmful to ones who are immunologically fragile like children and elders (Clare et al., 2003; Abbas et al., 2010; Abrantes et al., 2014). For the detection of H_2O_2 in milk, Wei et al. (2022) developed CQDs rich in phenolic hydroxyl groups that were synthesized in one step as a fluorescent probe. A glucose-oxidase (GOx) strategy was implemented for the quantitative analysis of H_2O_2. Due to the presence of phenolic hydroxyl groups, these CQDs exhibited fluorescence quenching at 510 nm upon oxidation by H_2O_2. The electron-donating characteristic of hydroxyl groups improved the reactivity of hydrogen atoms, allowing the CQDs to respond to hydrogen peroxide in milk via fluorescence quenching. The study further showed that fluorescence of CQDs got quenched at concentration levels of 1 to 100 µM H_2O_2. The probe was able to quantify the said adulterant even at very minute quantities of about 0.175 µM (Wei et al., 2022).

Adulteration with Glucose

Lactose is the predominant carbohydrate present in milk. Apart from lactose, monosaccharaides such as glucose and galactose are also present in milk in minute quantities. Various set of carbohydrates like glucose, have been intentionally added into milk, to earn more profit by enhancing the density and viscosity of milk (Nascimento et al., 2017). This glucose can have various negative health implications, henceforth, measuring the amount of glucose in milk is imperative in protecting the health of the general population. Wei et al. (2022) developed CQDs rich in phenolic hydroxyl groups to detect the concentration of glucose in milk directly. The CQDs were synthesized in one step as a fluorescent probe. Glucose oxidase (GOx)-mediated strategy was again used here for the detection of glucose. The authors reported that fluorescence of CQDs got quenched at concentration range of 10 to 100 µM of glucose. The probe was able to detect very minute quantities of glucose, even as low as 0.686 µM.

Detection of Vanillin

Vanillin is commonly added as flavouring agent in foods such as milk powder, ice cream, cookies, custard, pudding, chocolate, and drinks. Majority of commercial vanillin is synthetic and might induce nausea and headaches (Kouhi et al., 2022). As such, incorporation of vanilla flavour in infant formula is restricted for infants aged less than six months (Hui et al., 2023). Using platinum nanoparticle-serine-functionalized and boron-doped graphene quantum dot composite (Pt-Ser-B-GQD), Hui and co-authors, developed an electrochemical sensor with high sensitivity, selectivity, and stability, to detect vanillin in baby formula.Differential pulse voltage (DPV) was utilized herein presence of the sensor to detect vanillin due to its high sensitivity and selectivity. This resulted in hydrolysis of vanillin, which led to the formation of 3,4-dihydroxybenzaldehyde. Emergence of this aforesaid component resulted in a strong electrochemical signal, which was detected by the developed Pt-Ser-B-GQD sensor (Hui et al., 2023). The content of vanillin in infant formula, as measured by Pt-Ser-B-GQD sensor (1.28 ± 0.11) and Gas Chromatography-Mass Spectrometry (GC-MS) (1.31 ± 0.22), were almost same, according to Huang et al. (2007). This proved the high efficacy of the developed sensor.

Detection of Thiamphenicol

Thiamphenicol is widely used as a medicine for treating cattle, even lactating cows. However, the drug enters milk through the animal's body, eventually passing to humans, which can result in antibiotic resistance. Therefore, presence of the said drug is restricted in milk and milk products. In order to detect thiamphenicol in milk, a QD based optosensor was made by Sa-Nguanprang and his co-workers. For development of the optosensor, a mesoporous CQD-molecularly imprinted polymer (MPC-QDs-MIP) nanocomposite was combined with mesoporous carbon, tetraethyl orthosilicate and 3-aminopropyl triethoxysilane with QDs. As and when thiamphenicol got bound, reduction in fluorescence emission at 640 nm was reported from the MPC-QDs-MIP optosensor. The optosensor exhibited great selectivity and high sensitivity over structural analogues. It resulted in 93.5–100.1% recoveries with relative standard deviation below 5%, when tested on spiked milk. The optosensor was perfect for thiamphenicol analysis in food since it provided quicker, cheaper, and solvent-free detection while matching HPLC in accuracy (Sa-Nguanprang et al*.,* 2022).

Detection of Oxytetracyclin

Oxytetracycline (OTC) is a typical broad-spectrum tetracycline antibiotic used in animal husbandry and aquaculture to treat bacterial infections and to promote growth of animals (Chafer-Pericas et al*.,* 2011; Kim et al*.,* 2014*;* Su et al*.,* 2022). However, misuse or abuse may leave behind OTC residues in milk, meat, and eggs, which can lead to health risks such as liver damage, allergic reactions, and antibiotic resistance (Yan et al., 2015; Li et al., 2019; Zhou et al., 2019; Huahua et al., 2023). To prevent such incidents, regulatory bodies have set maximum residue limits (MRLs). World Health Organization (WHO) has set MRL as 0.1 µg/mL for OTC in milk (Wang et al*.,* 2018). This demonstrates how crucial it is to have a rapid, accurate, and reliable method of identifying OTC in food in order to safeguard human health and safety.

Guo et al. (2024*)* developed a dual-response fluorescence sensing platform based on molybdenum sulfide quantum dots (MoS_2 QDs) and europium ions (Eu^{3+}) for ratiometric detection of OTC. Here, water-soluble MoS_2QDs were produced via a hydrothermal technique with several modifications as compared to the research carried out by Wang et al.(2014). The preparation method of aforesaid QDs is underlined herewith. At first, the pH of the mixture was lowered to 6.5 using 0.1 M HCl after 0.25 g of NaO_4; $2H_2O$ was dissolved in 25 mL of deionized water and ultrasonically stirred for five minutes. In addition, about 0.5 g of L-cysteine and 50 mL of deionized water were added, and the mixture was homogenized using ultrasonography for ten minutes. The mixture was then transferred to a 100 mL Teflon-lined autoclave and heated to 200°C for 36 hours. After cooling, it underwent 20-minute centrifugation at 12,000 rpm, followed by which it passed through a 0.22 µm membrane filter. Consequently, for further purification, it was subjected to twenty four hour dialyzation treatment at 1000 Da MWCO (molecular weight cut off).Finally, the prepared quantum dots were stored at 4°C for further use. The developed setup was highly selective and sensitive towards OTC and was able to quantify even very trace quantities of OTC, as low as 2.21 nM. As the concentration of OTC increased, the emission at 624 nm increased and the fluorescence at 420 nm decreased. When exposed to UV light at 365 nm, the fluorescence colour changed from blue to red, becoming detectable to the naked eye (Guo et al*.,* 2024).

Conclusions:

As already stated, adulterated milk is a serious challenge for both manufacturer and consumer. Rapid and economical adulteration tests have already been devised to address the above challenge, but they too have limitations. As discussed in this paper QDs can play a prominent part in this regard. Not only they are capable to detect trace quantity of adulterant in the sample, but also they are rapid. In this article we have compiled the application of different QDs in detecting urea, formaldehyde, melamine, glucose and hydrogen

peroxide. In addition, we have also talked upon the usage of QDs in detecting certain anti-biotics and restricted flavouring constituents in milk and milk based products. Although, QDs depict very promising results, but further research is required to validate the usage of QDs in detecting few adulterants.

Conflict of interest:

The authors declare no conflict of interest.

References:

Abbas ME, Luo W, Zhu L, Zou J, Tang H. Fluorometric determination of hydrogen peroxide in milk by using a Fenton reaction system. Food Chemistry. 2010; 120 (1):327–31. https://doi.org/10.1016/j.foodchem.2009.10.024.

Abrantes MR, da SilvaCampêlo C, da SilvaJBA. Fraudeemleite: métodos de detecção e implicações para o consumidor.Revista do Instituto Adolfo Lutz. 2014; 73(3): 244–51. https://doi. org/10.18241/0073-98552014731611.

Acunha T, Ibáñez C, García-Cañas V, Simó C, Cifuentes, A. Recent advances in the application of capillary electromigration methods for food analysis and Foodomics. Electrophoresis. 2016; 37(1): 111-41.

Alanazi AZ, Alhazzani K, Mostafa AM, Barker J, Ibrahim H, El-Wekil MM, Ali AMBH. A novel urease-assisted ratiometric fluorescence sensing platform based on pH-modulated copper-quenched near-infrared carbon dots and methyl red-quenched red carbon dots for selective urea monitoring. Microchimica Acta. 2024; 191(8): 505.

Azad T, Ahmed S. Common milk adulteration and their detection techniques. International Journal of Food Contamination. 2016; 3: 22. https://doi.org/10.1186/s40550-016-0045-3.

Bonilla JC, Bozkurt F, Ansari S, Sozer N, Kokini JL. Applications of Quantum Dots in food science and biology. Trends in Food Science and Technology. 2016; 53:75-89.

Chafer-Pericas C, Maquieira A, Puchades A, Miralles J, Moreno A. Multiresidue determination of antibiotics in feed and fish samples for food safety evaluation. Comparison of immunoassay vs LC-MS-MS. Food Control. 2011; 22(6):993–9.

Chi SX, Liu BH, Zhang B, Wang BR, Zhou J, LiL, Mu ZS. Development of an ELISA method to determine adulterated cow milk in camel milk. International Dairy Journal. 2024;155:105953.

Choudhary S, Joshi B, Joshi A. Translation of carbon dot biosensors into an embedded optical setup for spoilage and adulteration detection. ACS Food Science and Technology. 2021; 1(6):1068-76.

Clare DA, Catignani GL, Swaisgood HE. Biodefense properties of milk: the role of antimicrobial proteins and peptides. Current Pharmaceutical Design. 2003;9(16):1239–55. https://doi.org/10.2174/1381612033454874.

Dai H, Shi Y, Wang Y, Sun Y, Hu J, Ni P, Li Z. A carbon dot based biosensor for melamine detection by fluorescence resonance energy transfer. Sensors and Actuators B: Chemical. 2014; 202: 201-8.

de la Fuente MA, Juarez M. Authenticity assessment of dairy products. Critical Reviews in Food Science and Nutrition. 2005; 45(7-8): 563–85. https://doi.org/10.1080/10408690490478127.

Demirhan BE, Demirhan B, Kara HES. Room-temperature phosphorescence determination of melamine in dairy products using l-cysteine-capped Mn-doped zinc sulfide (ZnS) quantum dots. Journal of Dairy Science. 2015; 98(5): 2992-3000.

Deng L, Li A, Gao Y, Shen T, Yue H, Miao J, Li R, Yang J. Detection of the bovine milk adulterated in camel, horse, and goat milk using duplex PCR. Food Analytical Methods. 2020; 13: 560-7.

Fathima Anjila PK, Tharani GR, Sundaramoorthy A, Shanmugam VK, Subramani K, Chinnathambi S, Pandian GN, Raghavan V, Grace AN, Ganesan S, Rajendiran M. An ultra-sensitive detection of Melamine in milk using Rare-earth doped Graphene Quantum Dots-Synthesis and Optical Spectroscopic approach. Microchemical Journal. 2024; 196: 109670.

Guo M, Bi M, Zhang F, Ye X, Ma P, Gao D, Song D. A dual-response ratiometric fluorescent sensor for oxytetracycline determination in milk and mutton samples. Talanta. 2024; 277:126382.

Huahua Z, Ruiyi L, Zaijun L. Excitation-depended fluorescence emission of boron-doped graphene quantum dot as an optical probe for detection of oxytetracycline in food and information encryption patterns, Microchimica Acta. 2023; 190(7): 278.

Huang CP, Li YK, Chen TM. A Highly Sensitive System for Urea Detection by Using CQDse/Zns Core-Shell Quantum Dots. Biosensors and Bioelectronics. 2007; 22(8):1835−8.

Hui J, Ruiyi L, Zhenzhong D, Xiaoyan G, Junshan X, Jinsong S, Zaijun L. Platinum nanoparticle-graphene quantum dot nanocage as a promising Schottkyheterojunctionelectrocatalyst for

electrochemical detection of vanillin in baby milk powder. Microchemical Journal. 2023; 186:108320.

HuX, Shi J, Shi Y, Zou X, Arslan M, Zhang W, Huang X, Li Z, Xu Y. Use of a smartphone for visual detection of melamine in milk based on Au@Carbon quantum dots nanocomposites. Food Chemistry. 2019; 272: 58–65. https://doi.org/10.1016/j.foodchem.2018.08.021

Joolaei Ahranjani P, Dehghan K, Esfandiari Z, Joolaei Ahranjani P. A Systematic Review of Spectroscopic Techniques for Detecting Milk Adulteration. Critical Reviews in Analytical Chemistry. 2025; 10: 1-32.

Juven BJ, Pierson MD. Antibacterial effects of hydrogen peroxide and methods for its detection and quantitation, Journal of Food Protection. 1996; 59: 1233–41. https://doi.org/ 10.4315/0362-028X-59.11.1233.

Kamthania M, Saxena J, Saxena K, Sharma DK. Milk Adulteration: Methods of Detection &Remedial Measures. International Journal of Engineering and Technical Research. 2014; 1: 15-20.

Kim CH, Lee LP, Min JR, Lim MW, Jeong SH. An indirect competitive assay-based aptasensor for detection of oxytetracycline in milk. Biosensors and Bioelectronics. 2014; 51: 426–30.

Kokkinos C, Angelopoulou M, Economou A, Prodromidis M, Florou A, Haasnoot W, Kakabakos S. Lab-on-a-membrane foldable devices for duplex drop-volume electrochemical biosensing using quantum dot tags. Analytical Chemistry. 2016; 88(13): 6897-904.

Kouhi I, Parvizi FardG, Alipour E, Saadatirad A.Development of an electrochemical sensor for determination of vanillin in some food stuffs. Journal of Food Processing and Preservation. 2022; 46(2): e16289.

Li C, Li J,Liang A, Wen G, Jiang Z. Aptamer turn-on SERS/RRS/fluorescence tri-mode platform for ultra-trace urea determination using Fe/N-doped carbondots, Frontiers in Chemistry. 2021; 9: 613083. https://doi.org/10.3389/fchem. 2021.613083.

Li C, Zhu L, Yang W, HeX, Zhao S, Zhang X, Tang W, Wang J, Yue T, Li Z. Amino-functionalized Al-MOF for fluorescent detection of tetracyclines in milk. Journal of Agricultural and Food Chemistry. 2019; 67(4): 1277–83.

Li L, Wu G, Hong T, Yin Z, Sun D, Abdel-Halim ES, Zhu JJ. Graphene quantum dots as fluorescence probes for turn-off sensing of melamine in the presence of Hg2+, ACS Applied Materials and Interfaces. 2014; 6(4): 2858–64. https://doi.org/10.1021/am405305r.

Livas D, Trachioti M, Banou S, Angelopoulou M, Economou A, Prodromidis M, Petrou P, Kakabakos S, Kokkinos, C. 3D printed microcell featuring a disposable nanocompositeSb/Snimmunosensor for quantum dot-based electrochemical determination of adulteration of ewe/goat's cheese with cow's milk. Sensors and Actuators B: Chemical. 2021; 334: 129614.

Ma P, Liang F, Sun Y, Jin Y, Chen Y, Wang X, Zhang H, Gao D, Song D. Rapid determination of melamine in milk and milk powder by surface-enhanced Raman spectroscopy and using cyclodextrin-decorated silver nanoparticles. Microchimica Acta. 2013; 180 :1173-80.https://doi.org/10.1007/s00604-013-1059-7.

Mostafapour S, Gharaghani FM, Hemmateenejad B. Converting electronic nose into opto-electronic nose by mixing MoS_2 quantum dots with organic reagents : application to recognition of aldehydes and ketones and determination of formaldehyde in milk. Analytica Chimica Acta. 2021; 1170: 338654. https://doi.org/10.1016/j.aca.2021.338654.

Murugesan P, Libiya N, Moses JA, Anandharamakrishnan C. Fluorescence resonance energy transfer-based sensor with silver-conjugated orange peel waste-derived carbon dots for melamine detection. Carbon Letters. 2023; 33(7): 2335-48.

Nagraik R, Sharma A, Kumar D, Chawla P, Kumar AP. Milk adulterant detection: Conventional and biosensor-based approaches. Sensing and Bio-Sensing Research. 2021; 33: 100433.

Nascimento CF, Santos PM, Pereira-Filho ER, Rocha FRP. Recent advances on determination of milk adulterants. Food Chemistry. 2017; 221: 1232-44.

Padilha JDS, Pedrozo-Peñafiel MJ, Azevedo MF, De Falco A, Larrudé DR, da Costa MEM, Aucélio RQ. Silver-modified nitrogen-doped graphene quantum dots as a sensor for formaldehyde in milk using headspace micro-extraction on a single-drop of aqueous nanoparticles dispersion. Analytica Chimica Acta. 2022; 1232: 340479.

Pang, S. A pH Sensitive Fluorescent Carbon Dots for urea and urease detection. fullerenes, nanotubes and carbon nanostructures. 2020; 28(9): 752−60.

Pérez-López B, Merkoci A. Nanomaterials based biosensors for food analysis applications. Trends Food Science and Technology. 2011; 2: 625-39.

Poonia A, Jha A, Sharma R, Singh HB, RaiAK, Sharma N. Detection of adulteration in milk: A review.

International Journal of Dairy Technology. 2016; 70(1): 23-42.

Sa-Nguanprang S, Phuruangrat A, Bunkoed O. An optosensor based on a hybrid sensing probe of mesoporous carbon and quantum dots embedded in imprinted polymer for ultrasensitive detection of thiamphenicol in milk. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2022; 264:120324.

Shalileh F, Sabahi H, Dadmehr M, Hosseini M. Sensing approaches toward detection of urea adulteration in milk. Microchemical Journal. 2023; 193: 08990.

Shi X, Wei W, Fu Z, Gao W, Zhang C, ZhaoQ, Deng F, Lu X. Review on carbon dots in food safety applications. Talanta. 2019; 194: 809-21.

Srivastava AK, Dev A, Karmakar S. Nanosensors and nanobiosensors in food and agriculture. Environmental Chemistry Letters. 2017; 16: 161-82.

Su P, Yu L,Ai Y, Zhang S, Ge H, Bu Y, Huang D, Wang X, Wang S. Conformational fixation induced fluorescence turn-on of oxytetracycline coordinated on aluminum-based metal-organic frameworks for ultrasensitive sensing application. Sensors and Actuators B: Chemical. 2022; 368: 132043.

Tudor Kalit M, Marković K, Kalit S, Vahčić N, Havranek J. Application of electronic nose and electronic tongue in the dairy industry. Mljekarstvo: časopiszaunaprjeđenjeproizvodnje i preradem lijeka. 2014; 64(4): 228-44.

Üzek R, Sari E, Denizli A. Detection of melamine by using fluorescent nanocomposites with specific recognition sites, chemistry select. 2021; 6(9): 2149–55. https://doi.org/10.1002/slct.202100130.

Valdes MG, ValdesGonzalez AC, Garcia Calzon JA, Diaz-Garcia ME. Analytical nanotechnology for food analysis. Microchimica Acta. 2009; 166: 1-19.

Wang Y, Ni P, Jiang S, Lu W, Li Z, Liu H, Li Z. Highly sensitive fluorometric determination of oxytetracycline based on carbon dots and Fe_3O_4 MNPs. Sensors and Actuators B: Chemical. 2018; 254: 1118-24.

Wang Y, Ni Y. Molybdenum disulfide quantum dots as a photoluminescence sensing platform for 2,4,6-trinitrophenol detection.Analytical Chemistry. 2014; 86(15): 7463–70.

Wei Y, Li L, Ma C, Wu Y, Zhu C, Gao H,Gu J, Xiong Y, Li X, Wang Z, Chen, G. Phenolic hydroxyl group–carbon dots as a fluorescent probe for the detection of hydrogen peroxide and glucose in milk. Journal of Applied Spectroscopy. 2022; 89(2): 272-80.

Xiao-Yue YU, Zi-Jun ZH, Yong-Mei WU, Yan LI, Jin-Cai LI, Yan-Hong BA, Jian-Long WA. Application Progress of Fluorescent Carbon Quantum Dots in Food Analysis. Chinese Journal of Analytical Chemistry. 2020; 48(10): 1288–95.

Xue G, Zhiying M, Xiuying L, Lijun T, Jianrong L. A Fluorescence Resonance Energy Transfer Biosensor Based on Graphene Quantum Dots and Protoporphyrin IX for the Detection of Melamine, Journal of Fluorescence.2020; 30: 1463–8, https://doi.org/10.1007/s10895-020-02524-z.

Yan Y, Liu Y, Yang J, Zhang J. A cathodic "signal-off" photoelectrochemicalaptasensor for ultrasensitive and selective detection of oxytetracycline. Analytical Chemistry. 2015; 87(24): 12215–20.

Yin W, Zhang Y, Gu J, Wang T, Ma C, Zhu C, Li L, Yang Z, Zhu T and ChenG. Urea detection in milk by urease-assisted pH-sensitive carbon dots. Applied Optics. 2021;60(33): 10421-8.

Zhao A, Chen Z, Zhao C, Gao N, Ren J,Qu X. Recent advances in bioapplications of C-dots. Carbon. 2015; 85: 309–27. https://doi.org/10.1016/j.carbon.2014.12.045.

Zhou N, Ma Y, Hu B, He L, Wang S, Zhang Z,Lu S. Construction of Ce-MOF@COF hybrid nanostructure: Label-free aptasensor for the ultrasensitive detection of oxytetracycline residues in aqueous solution environments. Biosensors and Bioelectronics. 2019; 127: 92–100.

***Corresponding author's email ID:** parthawbuafs16@gmail.com

Citation: Banerjee P, Chakraborty P, Debnath A, Chakraborty C, Roy PK, Kalla AK, Manik S, Debnath PP.Quantum Dots in Milk Adulteration. Indian Journal of Veterinary Public Health. 2025; 11(1): 7-15.

DOI: https://www.doi.org/10.62418/ijvph.11.1.2025.7-15