Paper Based Biosensor for the Detection of Antibiotic Residues in Milk

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Supervisor: Dr. Sobia Asghar

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AUTHOR'S DECLARATION

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DEDICATION

I am thankful to Allah Almighty who has always been my source of peace, and comfort. Allah has helped me throughout this journey and gave me the opportunity to pursue the master's degree at NUST. This is also dedicated to my parents, teachers, and my friends who supported me during this journey.

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

µPAD	Microfluidic Paper-based device
MOF	Metal Organic Framework
SEM	Scanning Electron Microscope
FTIR	Fourier Transform Infrared Spectroscopy
XRD	X-ray Diffraction
WHO	World Health Organization
FAO	Food and Agricultural Organization
Cu-btc	Copper benzene 3,5 Tricarboxylic acid
MRL	Maximum Residue Limit
LOD	Limit of Detection
ELISA	Enzyme-linked immunosorbent assay
DMF	N, N- Dimethyl formamide
OTC	Oxy tetracycline
TC	Tetracycline

ABSTRACT

The present study investigates the development and validation of a novel paper-based biosensor for the detection of antibiotic residues, specifically tetracycline, in milk, which is crucial for ensuring quality control in the dairy sector, a key component of agribusiness management. The effective management of antibiotic residues is vital not only for public health but also for maintaining consumer trust and compliance with international safety standards, which directly impacts the sustainability and profitability of the dairy industry. The increasing prevalence of antibiotic residues in dairy products, due to the overuse and misuse of antibiotics in livestock, poses significant public health risks, including the development of antibiotic-resistant bacteria. To address this challenge, a cost-effective, portable, and environmentally friendly sensor was designed using Whatman filter paper and a Metal-Organic Framework (Cu-MOF) with peroxidase-like activity. The sensor operates on a colorimetric principle, where the presence of tetracycline inhibits the oxidation of tetramethylbenzidine (TMB) by hydrogen peroxide, resulting in a quantifiable color change.

The Cu-MOF was synthesized and characterized using Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), and Fourier Transform Infrared Spectroscopy (FTIR), confirming its successful formation and appropriate chemical structure. The sensor's sensitivity was tested across a range of tetracycline concentrations, achieving a detection limit of 0.1 μM, which is comparable to existing detection methods. The paper-based device demonstrated excellent stability over time and maintained its functionality under various storage conditions.

This research highlights the potential of the developed sensor as a reliable tool for on-site detection of antibiotic residues in milk, contributing to food safety and public health. The sensor's ease of use, low cost, and effectiveness make it suitable for deployment in resource-limited settings, offering a practical solution to the widespread issue of antibiotic contamination in dairy products. Future work will focus on expanding the sensor's capabilities to detect other antibiotic classes and integrating a mobile application for real-time data analysis. This innovation not only ensures

consumer safety but also supports the agribusiness sector in maintaining high standards of product quality and market competitiveness.

Key words: Agribusiness Management, Sustainability, Filter paper, Copper metal organic framework, Innovation, Antibiotic residues, Tetracycline, Dairy industry, Agriculture, Quality Assurance, Food safety, Biological sensor.

CHAPTER 1

1 Introduction

Food-producing animals, such as dairy cows, have been administered antibiotics as part of disease control and regular well-being since the early 1930s (Patari et al., 2022).Antibiotics, also known as antibacterials, are synthetic/semi-synthetic chemical compounds that retard or eliminate the growth of bacteria**.** (Seymour et al., 1988) Statistics from the Centre for Disease Control and Prevention (CDC) as of 2020 reveal that at least 2.8 million people have developed significant drug resistance, of which 35,000 lives have been claimed. Some of the most common classes of antibiotics administered to dairy cows are aminoglycosides, tetracyclines and fluoroquinolones. Antibiotics act by penetrating the bacterial cells, altering their overall permeability and resulting in cell lysis.

Although antibiotics have significantly reduced disease occurrences and increased feed efficiency, their widespread use has raised serious public health concerns over the years (Mesgari Abbasi et al., 2011). To cope with increasing milk demand, practices of antibiotic administration to cows in the form of IV injections and regular feed additives has led to its over-use and misuse over the years. These pharmacologically active metabolites, known as “residues”, accumulate in the body of the animal over time. (Dror, D.K. and Allen, L.H. (2011) .When a drug is administered to the cow, it is broken down by the body. Most parts of the antibiotics get absorbed into the bloodstream (bioavailability), while the rest is excreted in the form of urine or feces, depending upon the animal itself and the dose provided. However, constant exposure over time results in the animal being antibiotic resistant, thus requiring higher doses to overcome the illness(Mesgari Abbasi et al., 2011). This can reflect in the antibiotics being present in animal products such as milk, eggs and meat, which becomes a consequential public health concern (Frontiers | Prudent Use of Antibiotics in Dairy Cows: The Nordic Approach to Udder Health, n.d.)

Tetracyclines (TCs) including Tetracycline (TC), Oxytetracycline (OTC) and Chlortetracycline (CTC) are broad-spectrum antibiotics widely used in animal husbandry for both prevention and treatment of diseases and feed additives to promote growth. In food-producing animals, tetracyclines may be administrated orally through feed or drinking water, parenterally, or by intra-mammary infusion. Due to entero-hepatic circulation, a small amount of administrated dosage may persist in the body for a long time after administration. (Nehra, Kumar, et al., 2023) The rate of metabolism of TCs in dairy cows has been estimated 25-75%. Tetracyclines in milk potentially may stain teeth of young children.(Vercelli et al., 2023)

Milk is a versatile and wholesome food that has been established as a great source of essential nutrients for centuries. It is fortified with appropriate amounts of fat, protein and vitamins, and it is extensively consumed as itself or its by-products. (Bovine - an Overview | ScienceDirect Topics, n.d.) Annual milk production during 2021/2022 was estimated approximately 65.7 million tons, giving Pakistan a place in the list of world's top 5 milk producing countries.(Blog, 2020). Being so widely consumed, it becomes imperative to monitor the amount of antibiotics present in milk before it reaches the consumer. Therefore, to overcome the above-mentioned challenges, the focus of this presented work was to design and develop a quick and reliable detection system to determine levels of antibiotics such as tetracycline in milk.

1.1 Rationale of the Study

Liquid chromatography-mass spectroscopy (LC-MS) has been considered the "gold standard" for the detection of antibiotics in milk. However, its long process time and analysis cost per sample has had researchers looking for a cheaper and more effective alternative.(Ghimpețeanu et al., 2022) With the advent of molecular technique such as antibodies and DNA, biosensors have garnered acclaim for their quick response time and sensitivity.(Antibiotic Residues in Food: Extraction, Analysis, and Human Health Concerns | Journal of Agricultural and Food Chemistry, n.d.). This study used nanomaterials such as metal organic framework (MOFs), which have large surface area and a good binding ability. MOF-199 is a highly porous structure that is formed of a metal ion and an organic linker. Drug delivery, and chemical sensing are the properties of these nanomaterials. In this study, a paper-based sensor for the easy detection of tetracycline and its family has been fabricated and developed. Whereas these nanoparticles were adsorbed onto the paper for the colorimetric reaction. The peroxidase-like activity of the as-fabricated Cu-btc was examined by TMB oxidation in the presence of H2O2. Tetracycline, possessing multiple N- and O-functional groups, acted as a potential electron donor to coordinate to Cu metal ions.(Martinez et al., 2010) Therefore, the Cu-btc-catalyzed color reaction was inhibited with the addition of tetracycline due to the formation of tetracycline–Cu complexes, resulting in a color change from green (oxidized TMB) to light brownish/colorless (TMB). Herein, because of this, a colorimetric assay based on

Cu-btc was designed for the detection of tetracycline residues in water samples and milk. This application of a paper substrate aims at the feasibility of having an on-farm, cost- effective, point-of-care device for screening of antibiotics. This device would also help veterinarians and farmers to make mindful decisions on administering antibiotics to cows. Furthermore, to evaluate its deploy ability and the extent of color development, spectroscopic and camera-based image processing techniques were performed on this sensor.

1.2 Objectives

Pakistan ranks among five top countries in the terms of milk consumption. Although, Milk is a healthy food and has numerous benefits for the consumers but almost seventy percent (70%) of the food-borne diseases are milk-based. Food adulterants like (urea, starch, detergents, oil, sodium carbonate) and Antibiotic residues present in milk causes illnesses and various diseases in milk consumers. The antibiotic residues (veterinary injections) which are being injected to the animals to increase their growth size and to make them look bulky are causing antibiotic resistance in humans. According to the Express Tribute, Antibiotic resistance is causing 300,000 deaths in Pakistan annually. ("Antimicrobial Resistance Causing 300,000 Deaths Annually in Pakistan," n.d.). This situation is alarming and needs to be solved to combat the antibiotic resistance in humans. This study has proposed that by developing an easy solution to test milk will provide point of care diagnostics. The objective of this study is to make a portable, sustainable, and environmentally friendly device to test antibiotic residues. People can easily check the quality of milk at ease. This device is different from other detection techniques available in the market. It is cheap and easy to use. The uniqueness and competitiveness of this device is that it is user-friendly, portable, lightweight, biodegradable, and sustainable. Farmers, shopkeepers, and households can use it easily. The device is made up of filter paper. Nanomaterials are being inserted into the paper which reacts with the antibiotic and gives a colorimetric reaction. This solution has good impacts on society. It is not only providing point of care testing but also giving antibiotic awareness among people. Paper-based device is covering 13^{th} UN's sustainable development goal which is to provide good health and Well-being. The main objective of this study is to contribute towards food security and quality assurance. Herein, this study has developed a μPAD-based paper sensor using microfluidic technology for rapid discrimination of antibiotics such as tetracycline in different milk samples. The antibiotics with chelating properties can substitute the ligands in nanomaterials such as metal organic framework (MOFs). It results in a distinct color change, which could be subsequently detected by a mobile phone. The colorimetric patterns obtained by using a series of metal-ligand complexes provided a unique colorimetric signature for tetracycline. (Taghizadeh-Behbahani et al., 2022)

CHAPTER 2

2 Literature Review

Bovine milk is one of the most consumed foods in the world and it is important for its high nutritional value and for its key role in the worldwide economy. (The State of Food and Agriculture 2019 | FAO, n.d.) Its composition comprises proteins (3.0–3.9%), lactose (4.4–5.6%), fat (3.3–5.4%), and minerals (0.7–0.8%) (Roy, Ye, Moughan, & Singh, 2020). Milk is an important dietary source of a variety of micronutrients, including calcium, phosphorus, magnesium, zinc, iodine, potassium, vitamin A, vitamin D, vitamin B12, and vitamin B2 (Dror & Allen, 2014).Antibiotic therapy has been employed by farmers and veterinarians for over a decade in the treatment of infectious diseases of dairy cattle, the most common of which, undoubtedly, has been mastitis. In some instances, the drugs have been incorporated into feed as a dietary supplement. Such uses have led to the presence of antibiotic residues in milk and food products. One of the most important concerns about bovine milk is the contamination with veterinary medicaments: antibiotics and anti-inflammatory drugs are administered to dairy cows to treat pathologies such as mastitis, endometritis, bronchopathies, pneumonia and lameness (EMA & EFSA, 2017; Han et al., 2015). The treatment of animals is mandatory to respect animal welfare but must be performed in a rational way: focusing on antibiotics, a targeted therapy using narrow spectrum molecules should be preferred, for the shortest time necessary to achieve a favorable therapeutic outcome (Rajala-Schultz, Nødtvedt, Halasa, & Persson Waller, 2021). In the past, antibiotics were employed not only for the sole therapeutic purpose, but also to promote growth of food producing animals and in metaphylactic protocols (Lees, Pelligand, Giraud, & Toutain, 2021). Without stringent regulation, an overuse, or a misuse of antibiotics was widespread and, consequently, foodstuff contamination due to antibiotics and antibiotic residues was not unusual (Lees et al., 2021). The interest of the scientific community pushed several nations to change legislation to restrict antibiotic drug usage and preserve consumers' health (Lees et al., 2021; Luiz et al., 2018). European legislation (EU, 2019a) confirmed the ban of these substances as growth promoting agents and severely restricted prophylactic (the administration of antibiotics to prevent the occurrence of any diseases or infection, prior the onset of clinical signs of a disease), and metaphylactic uses (i.e., the treatment of a small group of clinically sick animals of other animals that could potentially develop the disease, and that might be sub clinically infected due to the strict contact to control the spread of the disease). European Union decided for this restriction already in 2006, anticipating the USA and China that banned the use of antimicrobial drugs as growth promoting agents only in 2017 and 2020, respectively (Lees et al., 2021**).**

The over-the-limit administration of veterinary antibiotic doses to food animals to prevent diseases, for feed intake proficiency and growth enhancement, is one of the major reasons for antibiotic misuse. (Sachi et al., 2019). Because of the potential toxic properties of antibiotic residues, the consumption of contaminated food with these residues establishes a direct risk for public health. Based on the Bayesian statistical model, it is estimated that the use of antibiotics in livestock will increase up to 200 235 tons by 2030. Moreover, it has also been reported that these antibiotic residues, due to their high stability, are not destroyed even during the thermal processing of food products. (Rayappa et al., 2023)

Antibiotic Classes	**Examples used in livestock**
Aminoglycosides	Gentamycin, neomycin
Macrolides	Erythromycin, tilmicosin, lincomycin, tulathromycin, tylosin
Tetracyclines	Chlortetracycline, oxytetracycline, tetracycline
Fluoroquinolones	Ciprofloxacin, danofloxacin, enrofloxacin
β-lactams	Penicillin, ceftiofur, amoxicillin
Sulphonamides	Various sulfonamides

Table 1: Various Veterinary Antibiotic Classes and Their Examples Used in Various Livestock and Animal Feed

Epidemiological and toxicological studies have concluded that the ingestion of antibiotic residues can result in increased health risks for all age groups resulting in immunopathological effects, carcinogenicity, mutagenicity, nephropathy, hepatotoxicity, reproductive disorders, and even chronic toxic effects due to prolonged low-level exposure.(Bacanlı & Başaran, 2019) Apart from these ill effects, exposure to these residues will in-directly give rise to antibiotic resistant bacteria with antibiotic-resistant genes that can cause greater harm to humankind.(Chen et al., 2019) This will also cause current antibiotic therapy to treat various diseases in humans to become a failure and the World Health Organization (WHO) has also declared it a global threat**.**(Menkem et al., 2018)

Among the various antibiotics, tetracycline is commonly employed to treat several bacterial infections in both animals and humans owing to its broad-spectrum antibacterial activity, low toxicity, good oral absorption, and low cost.(Zheng et al., 2020) After the absorption of tetracycline, approximately 30–90% of it is excreted in the environment through urine and excreta still as the parent compound. The excessive use and even misuse of tetracycline has led to increased levels of its residues in surface water, ground water, and drinking water along with animal products and particularly in milk. Moreover, the high hydrophobicity and low volatility of tetracycline causes it to remain in wastewater for 34 to 329 h These residues can have a negative impact on human health as well as the environment through a series of physicochemical and biological processes. Consequently, several countries have set maximum residue limits (MRLs) for tetracycline to ensure water and food safety, e.g., a limit of 100 mg L-1 in milk was set by the Food and Agriculture Organization, United Nations (FAO), and World Health Organization (WHO). Therefore, the routine monitoring of antibiotics, especially tetracycline residues, is of great significance in ensuring water and food safety. (Veterinary Drug Detail | CODEXALIMENTARIUS FAO-WHO, n.d.).

Currently, several approaches have been developed for the detection of antibiotics, such as the enzyme-linked immunosorbent assay (ELISA), Raman spectroscopy, surface plasma resonance (SPR) sensors, fluorometric detection, high performance liquid chromatography (HPLC), capillary electrophoresis, colorimetric sensors, and electrochemical detection. Nonetheless, while most of these conventional techniques offer high sensitivity and accuracy, they can only be performed in well-equipped laboratories by trained personnel.(Anh et al., 2022)

Biochemical sensors are seen as an alternative to these quantification methods since they are easy to handle, less expensive, portable, and rapid. There is an unmet need for development of simple and low-cost detection systems to provide unskilled users with the ability to easily detect analytes and evaluate results in resource-limited settings. (Cháfer-Pericás et al., 2010) So, Paper-based microfluidic approaches enable the fabrication of low-cost, simple, flexible, and portable diagnostic platforms .This simple technology uses paper as a substrate to create microfluidic channels by patterning hydrophobic materials on hydrophilic paper.(Grau, 2017). To test a biological substance (e.g., blood, urine, saliva, sweat, tear) or an environmental reagent (e.g., heavy metal ion, hydrogen sulfide gas) that contains an analyte of interest, the sample is applied to the device and wicked to a detection zone by capillary action without the need to use an external pump. (Guinati et al., 2021).Analyte detection from the sample is facilitated by a chemical reaction which induces a change in color, electrochemical properties and light absorption or emission. Among these methods, the most frequently used detection approach is based on colorimetric change.

2.1 Advantages of a paper-based sensor

The main advantages of paper include (i) high surface to volume ratio; (ii) adsorption properties; (iii) capillary action; (iv) compatibility with biological samples; (v) chemical functional groups for immobilization of proteins and antibodies. (Bioactive Paper Provides a Low-Cost Platform for Diagnostics - ScienceDirect, n.d.) Furthermore, the ability to store and transport reagents within the paper matrix eliminates the need for users to handle chemical solutions. In addition, paper is lightweight and accessible globally. Finally, paper-based microfluidic devices can be easily fabricated at a low-cost using practical fabrication techniques such as Hot-embossing (Lu et al., 2010)

2.2 Paper type

More recently, filter paper has been used in paper-based sensors which is also manufactured by Whatman (Maidstone, United Kingdom). This type of paper has been used for its relatively uniform thickness and wicking properties as well as superior adsorption and retention of reagents compared to similar types of paper. (Martinez et al., 2010)

2.3 Fabrication of paper-based devices

The fabrication method that is used to produce the biosensors can impact the simplicity and their applications. There are numerous approaches available that involve chemical modification or physical deposition onto the paper. In this study, Hot-embossing technique has been applied for the fabrication of paper. (Grau, 2017).

2.4 Advanced Nano Materials

Advanced nanomaterials have been used for the detection and quantification of low molecular weight organic and bio-macromolecular compounds and have eventually given rise to rapid, sensitive, portable, and on-site sensing choices. Recently, metal–organic frameworks (MOFs) have attracted enormous research interests as peroxidase-mimetic enzymes, such as MIL-88(Fe), MIL-53(Fe), MIL-68(Fe), and HKUST-1 (Cu-btc) (Zhang et al., 2023). MOFs are highly ordered porous structures that are formed by the self-assembly of metal ions and organic linkers. The diverse applications of MOFs in the field of chemical sensing, drug-delivery, catalysis, etc. are closely related to their potential features in terms of tunable pore size, structural diversity, large surface area, and excellent thermal stability.(Yusuf et al., 2022) The study reported the peroxidase-like activity of Cu-btc by the oxidation reaction of TMB with H2O2. Cu-btc, possessing a high catalytic activity and wide linear range, has potential to be utilized as an artificial enzyme mimic for application in biosensors catalysis, and the food industry. (Souhila et al., 2021) The peroxidase-like activity of the as-fabricated Cu-btc was examined by TMB oxidation in the presence of H2O2. Tetracycline, possessing multiple N- and O-functional groups, acted as a potential electron donor to coordinate to Cu metal ions(Younas et al., 2019) Therefore, the Cu-btc-catalyzed color reaction was inhibited with the addition of tetracycline due to the formation of tetracycline–Cu complexes, resulting in a color change from blue (oxidized TMB) to colorless (TMB). Based on this mechanism, a colorimetric assay based on Cu-btc was designed for the detection of tetracycline residues in water samples. In comparison to metal ions-based simple catalytic systems, Cu-MOF possesses superiority in terms of its higher catalytic activity at neutral pH than acidic pH.(Nehra, Madan, et al., 2023)

CHAPTER 3

3 Materials and Methods

3.1 Materials

Cu (NO3) 3H2O, trimesic acid (1,3,5-tricarboxylic acid), tetracycline, amoxicillin, penicillin, co-trimoxazole, streptomycin, and N,N-dimethylformamide (DMF) were procured from Sigma-Aldrich. Acetate buffer and hydrogen peroxide (H2O2) were obtained from a laboratory. All the chemicals in this study were of analytical grade and utilized without any further purification. Distilled water (DI) was also procured from a supplier.

3.2 Synthesis of MOF

According to a hydrothermal approach, 17 ml solution of Cu (NO3)2 3H2O (2 g) was prepared in DI water. Trimesic acid (1 g) was dissolved in a mixture of DMF and ethanol (1:1). Both precursors were mixed under ultrasonication separately for 5 min to obtain clear solutions. Thereafter, trimesic acid solution was dropwise added to the metal precursor under sonication. The final solution was processed in loosely capped reagent bottles over a magnetic stirrer at 100 1C for 41 h, The solution was then transferred to a 100 mL capacity Teflon-lined hydrothermal autoclave and heated at 100 1C for 41 h. The final product was processed in the same manner as above and named Cu-MOF2.

Figure 1: Cu-MOF synthesis process

3.3 Peroxidase-like activity

Cu-MOF was employed as the catalyst for the oxidation of TMB by H2O2 in acetate buffer. In detail, 2 mL of 0.1 mg mL1 CuMOF (dispersed in acetate buffer), 250 mL TMB (3 mM in DMF), and 250 mL H2O2 (6 mM) were mixed and reacted for 30 min at 25 1C. The formation of the TMB-oxidized product was confirmed through a color change.

a) Colorimetric detection of tetracycline using Cu-MOF

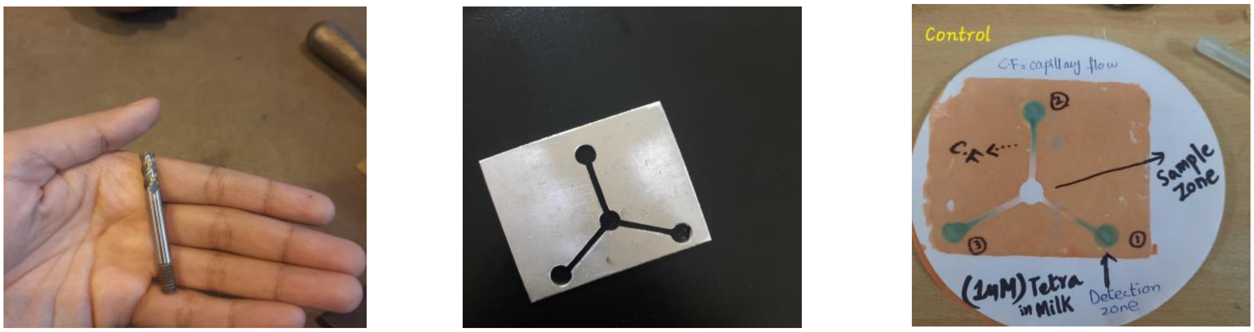
For the colorimetric detection of tetracycline, a 4 mL assay solution containing the same amounts of TMB, MOF, and H2O2 with varied concentrations of tetracycline was prepared. In detail, the solution contained 2 mL of 0.1 mg mL1 Cu-MOF2 (dispersed in acetate buffer), 250 mL TMB (3 mM in DMF), 250 mL H2O2 (6 mM), and 2 mL of varied concentrations of tetracycline (0.1, 0.3, 0.5, 0.7, 1, 5, 10, 25, 50, and 100 mM), respectively. The change in absorption spectra due to the varied concentration of tetracycline was recorded by UV-Vis spectrophotometry. For the selectivity study, 2 mL of interfering antibiotics were added instead of tetracycline

3.5 Preparation of sensor

To prepare a metal chip, Aluminum sheet of diameter 2.5 mm and End mill cutters of size 3mm from hardware shop were sent to an engineering workshop. Bees wax and crayons were procured from Daraz. For the fabrication of a paper sensor, Whatman filter paper and butter paper were obtained from stationery. Metal chip for hot embossing was prepared via drilling with end mill cutters. The depth was 3mm and the overall thickness of the chip was 2.5mm based on the calculations.

First, the filter paper was dropped into the melting wax for 10 seconds. Then it dried at room temperature. After drying the paper, the waxed filter paper was placed onto the simple filter paper and the metal chip was positioned onto them. The chip was heated first at 100-150º Celsius to get the proper stamp onto the paper. Pressure application was then given to the stamp placed over the paper with the help of 10 kilograms weight. The time required for the pressure application was 1.5 minutes. After this, it was released and separated and through this hot embossing technique, the proper design of the metal chip was fabricated onto the waxed filter paper. (Antibiotics and Nitrite Detection in Diagnostics with Paper-Based Microfluidics - YouTube, n.d.)

It consists of three detection zones and one sample zone. The milk/water sample was dropped onto the fabricated paper pad (μpad)'s sample zone which then travelled to the three detection zones with the help of a capillary action. Whereas specific nanomaterials were inserted into the detection zones for the colorimetric change. A quantity of 15 μL was kept in consideration while putting the sample onto the paper. (Kumar Rayappa et al., 2023)Whereas 25 µL nanomaterials were being inserted into the detection zones.



(a) (b) (c)

Figure 2: Microfluidic based paper sensor. (a): End mill cutter of diameter (3mm) (b): Aluminum Metal chip (c): Paper fabricated through hot-embossing technique

CHAPTER 4

4 Results

4.1 Stability of the Paper-Based Device

The stability of the paper-based device was assessed over a period of several weeks under ambient storage conditions. The device showed no significant degradation in performance or structural integrity, indicating that the materials and fabrication methods used were effective in creating a durable sensor. The results suggest that the paper-based device can be stored and used over extended periods without loss of functionality, making it a viable option for both laboratory and field applications. The metal chip was successfully fabricated and integrated into the sensor using the hot embossing technique. The metal chip's design allowed for precise and reproducible sample application and detection. The chip's durability and functionality were tested through repeated cycles of use, demonstrating that it can withstand the rigors of practical application without losing its structural integrity or performance capabilities.

4.3 Advanced Nanomaterial Characterization

The synthesized Cu-MOF was characterized using Scanning Electron Microscopy (SEM), X-ray Diffraction (XRD), and Fourier Transform Infrared Spectroscopy (FTIR). The SEM analysis revealed the octahedral crystal structure of the Cu-MOF, with varying sizes of crystals confirming the successful synthesis

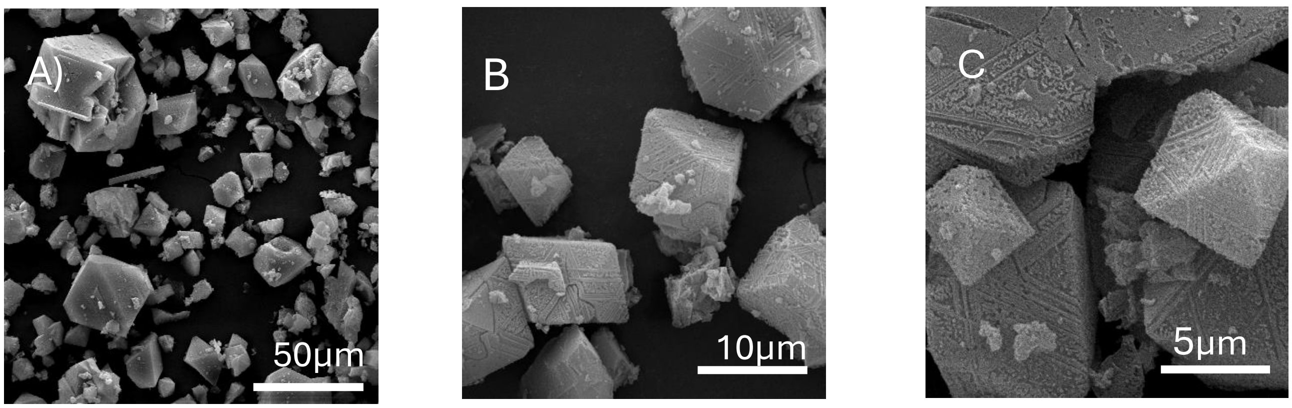
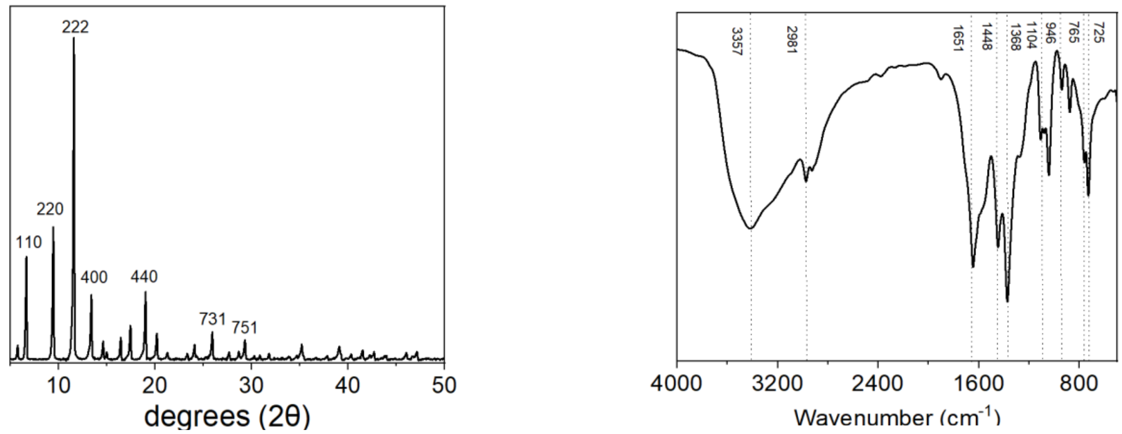


Figure 3: SEM analysis of Cu-MOF of varying sizes (a): 50μm (b): 10μm (c) 5μm

The XRD analysis of the Cu-MOF showed good matching with the literature, and it confirms the good crystallinity of Cu-MOF. The XRD pattern of the Cu-MOF exhibited distinct peaks at 2θ values of 6.4°, 9.8°, 11.64°, 13.48°, 14.7°, 25.92°, and 29.2°, corresponding to crystal planes (110), (200), (222), (400), (410), (440), (731), and (751). These peaks were consistent with the crystal planes reported in the literature This study confirms the effective formation of the Cu-MOF peaks that are sharp and higher intensity, demonstrating a well-integrated structure in which the MOF matrix mostly determines the composite's overall crystallinity.

The presence of different bonds present in the synthesized materials were confirmed by the FTIR Spectra of Cu-MOF is shown in the wavenumber range of 4000 cm^{-1} to 800 cm^{-1} as shown in Fig 4. The attachment of Cu to the benzene ring of BTC is responsible for the peaks at 729 and 760 cm^{-1}. The C-O-Cu bonds' stretching causes the bands at 940 and 1110 cm-1 to show up. The symmetric and asymmetric vibrations of Cu-O bonds are responsible for the bands at 729 and 760 cm^{-1}. Whereas a wide band at 3000–3600 cm^{-1} corresponds to the OH group's stretching vibrations.



(a)

(b)

Figure 4: XRD & FTIR patterns of the synthesized material respectively. (a) shows XRD peaks at different angles. (b): FTIR spectra shows the presence of different bonds in the material

4.4 Peroxidase Activity of MOF

The peroxidase-like activity of the synthesized Cu-MOF was evaluated using a colorimetric assay involving TMB and H_2O_2. The Cu-MOF catalyzed the oxidation of TMB in the presence of H_2O_2, resulting in a visible color change from colorless to blue. This activity was further inhibited in the presence of tetracycline, which binds to the Cu-MOF and prevents the oxidation of TMB. The results demonstrate the potential of Cu-MOF as a peroxidase mimic and its applicability in the colorimetric detection of tetracycline.

4.5 Colorimetric Detection of Tetracycline

The paper-based sensor was tested with varying concentrations of tetracycline to evaluate its sensitivity and detection limits. The color change intensity was measured using image analysis software Image J, and the results indicated a clear correlation between tetracycline concentration and color intensity. The sensor was able to detect tetracycline at concentrations as low as 0.1 µM, with a detection limit comparable to or better than other reported methods using similar nanomaterials. The detection range of the device was found to be 0.1-0.7 µM, which is suitable for the detection of tetracycline residues in milk.

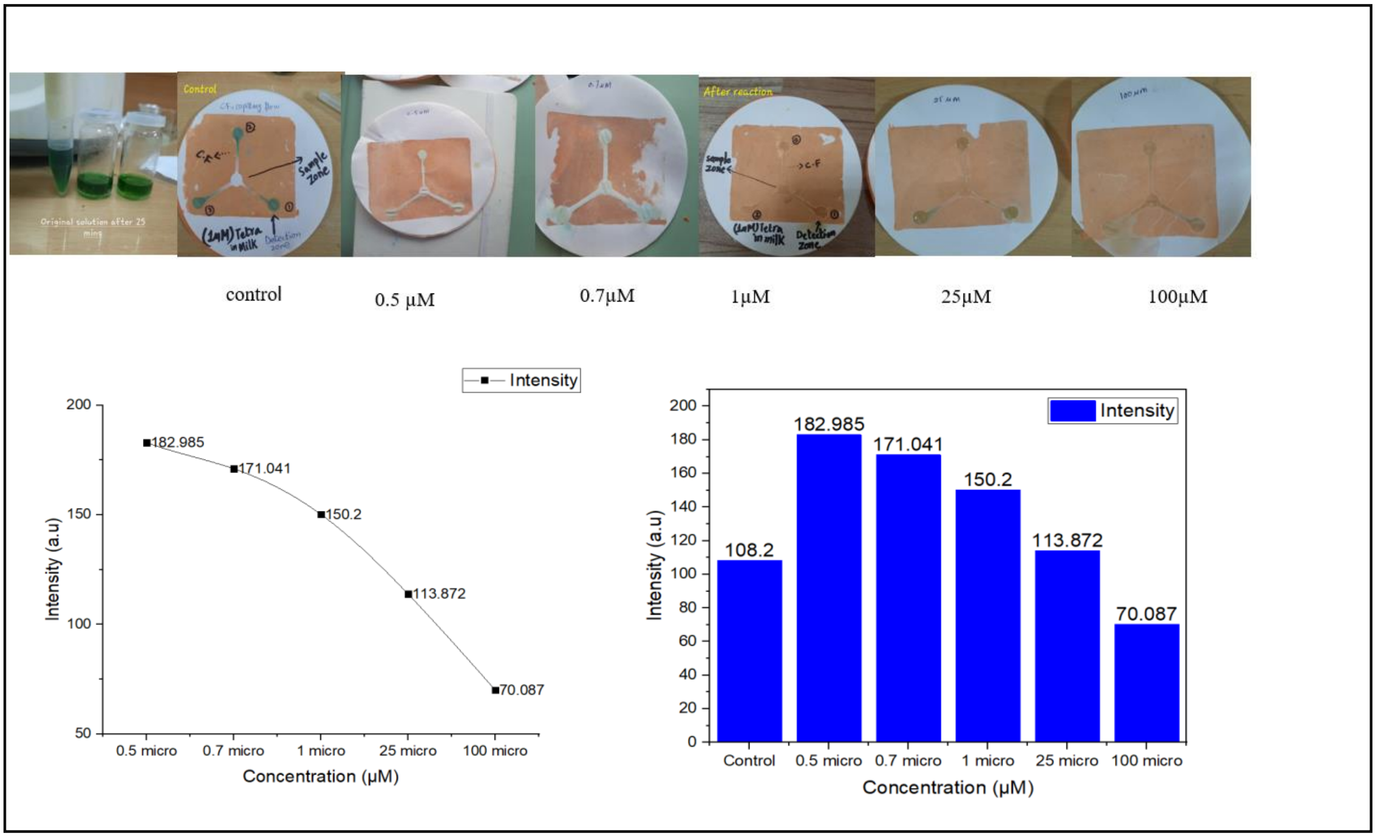


Figure 5: Image J analysis showing the color variation from white/colorless to brown with the concentration of 0.1 to 100 micromolar.

4.6 Optimization of the Sensor

The limit of detection (LOD) and detection range of the system were calculated according to the three times the signal to noise rule (S/N = 3) and were found to be 0.5 µM and 0.1 to 0.7 µM, respectively. The LOD of our system was not the lowest; however, its LOD was lower than the majority of the reported methods, refer to Table 2 . Moreover, the LOD of our TMB- H2O2/Cu-MOF2 system was also able to meet the requirements of US EPA and WHO. The optimization of the sensor involved adjusting the concentration of Cu-MOF, the volume of the sample applied, and the conditions for colorimetric detection. Through a series of experiments, the optimal conditions were determined to be a Cu-MOF concentration of 0.1 mg/mL, a sample volume of 25 µL, and an incubation time of 30 minutes. Under these conditions, the sensor provided the most accurate and reliable detection of tetracycline in milk samples.

S. NO.	MATERIAL	CATALYST LOADING (MG/ML)	DETECTION RANGE (µM)	LIMIT OF DETECTION (LOD) (µM)
1	**MIL (FE/CO)**	0.3	1-8	0.24 µM
2	**CARBON DOTS**	1.8	0-150	0.46 µM
3	**FE3O4**	0.1	2-225	0.4 µM
4	**GOLD NANOPARTICLES**	0.5	0.1-5	0.7 µM
5	**CU-MOF**	0.1	0.1-0.7	0.5µM

Table 2: Shows the comparison of detection range and LOD between Cu-MOF and other materials

CHAPTER 5

Discussion

The detection of antibiotic residues in milk is a significant public health concern due to the potential risks associated with the consumption of contaminated dairy products. The overuse and misuse of antibiotics in livestock, particularly in the dairy industry, have led to the presence of these residues in milk, contributing to the growing problem of antibiotic resistance. The development of a paper-based sensor for detecting these residues represents a critical advancement in ensuring food safety, particularly in regions where access to sophisticated laboratory equipment is limited.

In this study, the paper-based sensor was designed using Whatman filter paper and a Metal-Organic Framework (Cu-MOF) with peroxidase-like activity. The choice of materials was strategic, as Whatman filter paper is known for its uniform thickness, high absorption capacity, and compatibility with biological samples, making it an ideal substrate for the sensor. The Cu-MOF, with its high surface area, structural stability, and catalytic properties, was selected to facilitate the colorimetric detection of tetracycline, a common antibiotic residue found in milk.

The sensor operates on a simple yet effective principle: the presence of tetracycline inhibits the oxidation of tetramethylbenzidine (TMB) by hydrogen peroxide, leading to a visible color change. This colorimetric response was quantified using image analysis software, allowing for the detection of tetracycline at concentrations as low as 0.1 μM. The sensitivity of the sensor is comparable to, if not better than, traditional methods such as liquid chromatography-mass spectrometry (LC-MS), which are typically used for antibiotic residue detection. However, unlike LC-MS, the paper-based sensor is portable, cost-effective, and easy to use, making it accessible to a broader range of users, including farmers, veterinarians, and small-scale dairy producers.

The stability of the sensor was evaluated under various storage conditions, and it was found that the device maintained its functionality over extended periods without significant degradation. This durability is crucial for practical applications, as it ensures that the sensor can be stored and used as needed without requiring frequent replacement or recalibration. The reproducibility of the results further underscores the reliability of the sensor, making it a valuable tool for routine monitoring of milk quality.

One of the major advantages of the paper-based sensor is its potential for use in resource-limited settings. Traditional methods for detecting antibiotic residues often require expensive equipment, skilled personnel, and significant time investments, making them inaccessible to many dairy producers in developing countries. In contrast, the paper-based sensor can be deployed directly on-site, providing immediate results that can inform decisions about milk safety and quality. This immediacy is particularly important in the context of agribusiness management, where timely decision-making can prevent the distribution of contaminated products and protect public health.

Despite its many advantages, there are some limitations to the current design of the paper-based sensor. For example, while the sensor is effective at detecting tetracycline, its ability to detect other classes of antibiotics needs to be further explored. Additionally, the colorimetric response is currently limited to qualitative analysis; while it provides a clear indication of the presence of tetracycline, it does not offer precise quantification of the residue levels without further calibration. Addressing these limitations will be essential for expanding the applicability of the sensor to a wider range of antibiotics and ensuring that it can meet the stringent regulatory requirements for food safety testing.

Future Recommendations

Building on the findings of this study, several avenues for future research and development can be identified:

1. **Expansion to Other Antibiotics:** Future work should focus on adapting the sensor to detect a broader spectrum of antibiotic residues beyond tetracycline. This could involve modifying the Cu-MOF or exploring alternative nanomaterials that are selective for other antibiotic classes, such as aminoglycosides or sulfonamides.

2. **Quantitative Analysis**: While the current sensor provides qualitative results, the development of a system for quantitative analysis is recommended. This could involve the integration of a mobile application that can analyze the intensity of the color change

and provide an estimate of the antibiotic concentration. Such an application would enhance the sensor's utility for regulatory compliance and quality control.

3. **Field Trials:** To validate the sensor's performance in real-world conditions, extensive field trials should be conducted. These trials should include a variety of milk samples from different regions and production systems to assess the sensor's robustness and reliability across diverse environmental conditions.

4. **Integration with IoT Devices:** The future development of this sensor could also involve its integration with Internet of Things (IoT) devices for real-time monitoring and data collection. This would enable continuous surveillance of milk quality in large-scale dairy operations, allowing for immediate intervention if antibiotic residues are detected.

5. **Environmental Impact Assessment:** Given the increasing focus on sustainability, it is important to assess the environmental impact of the sensor, particularly in terms of its biodegradability and the lifecycle of the materials used. Future research should explore the use of more sustainable materials and the potential for recycling or safe disposal of the sensor after use.

6. **Regulatory Approval and Standardization:** Finally, efforts should be made to obtain regulatory approval for the sensor from relevant food safety authorities. This would involve demonstrating the sensor's compliance with international standards and ensuring that it meets the requirements for use in official testing protocols. Standardization of the sensor's production and use would also facilitate its widespread adoption in the dairy industry.

In conclusion, the development of this paper-based sensor represents a significant step forward in the detection of antibiotic residues in milk. By addressing the current limitations and pursuing the recommended areas of future research, this sensor has the potential to become a widely used tool for ensuring the safety and quality of dairy products, thereby supporting public health and sustainable agribusiness practices.

Conclusion

In the present work, a paper-based (Whatman filter paper grade 4) device is fabricated and used for detecting multiple (seven) adulterants in milk samples simultaneously based on the colorimetric technique. Observation reveals that only 1–2 mL of sample volume is required for each test, and the testing time is less than 30 s. Qualitative and quantitative analyses are carried out, with a recovery range of 85–107% and RSD range of 0.50–3.51% using the developed calibration curves. The adulterants' linear range, sensitivity, and LOD are close to the existing methods. The cost of testing for antibiotics is approximately Rupees five hundred to six hundred (Rs.500-600) only. The method is reliable for detecting antibiotics in milk on the spot. Further, the lightweight, low-cost, simple-to-use, and environmentally friendly method makes this device suitable for inspecting many liquid foods. It is inferred from the investigation that the reagent only reacts with the specific antibiotic (tetracycline) in this method and not with any milk ingredients. Hence, this analytical tool can help to monitor liquid food safety and thereby increases the traceability of tainted milk in remote areas of developing countries.

It should be noted that by appropriately modifying the chemical reagents, the current device can be used not only for milk but also for water, protein shakes, fruit juices, etc. Further, the future aim is to use a mobile application to perform quantitative analysis to determine the adulterants' concentration. Emphasis will also be given to addressing the possible limitation of the developed device, e.g., reagent evaporation effect, a common platform for finding the color intensity in different brightnesses, detecting any unknown adulterants using artificial intelligence, and so on.

References

- Anh, N. H., Doan, M. Q., Dinh, N. X., Huy, T. Q., Tri, D. Q., Loan, L. T. N., Hao, B. V., & Le, A.-T. (2022). Gold nanoparticle-based optical nanosensors for food and health safety monitoring: Recent advances and future perspectives. *RSC Advances*, *12*(18), 10950–10988. https://doi.org/10.1039/D1RA08311B
- *Antibiotic Residues in Food: Extraction, Analysis, and Human Health Concerns | Journal of Agricultural and Food Chemistry*. (n.d.). Retrieved August 18, 2024, from https://pubs.acs.org/doi/10.1021/acs.jafc.9b01334
- *Antibiotics and Nitrite detection in diagnostics with paper-based microfluidics—YouTube*. (n.d.). Retrieved August 18, 2024, from https://www.youtube.com/watch?v=KsvcUA-t4EY
- *“Antimicrobial resistance causing 300,000 deaths annually in Pakistan.”* (n.d.). Retrieved August 18, 2024, from https://tribune.com.pk/story/2467320/antimicrobial-resistance-causing-300000-deaths-annually-in-pakistan
- Bacanlı, M., & Başaran, N. (2019). Importance of antibiotic residues in animal food. *Food and Chemical Toxicology*, *125*, 462–466. https://doi.org/10.1016/j.fct.2019.01.033
- *Bioactive paper provides a low-cost platform for diagnostics—ScienceDirect*. (n.d.). Retrieved August 18, 2024, from https://www.sciencedirect.com/science/article/pii/S0165993609001307?via%3Dihub
- Blog, P. (2020, March 1). Milk Production in Pakistan. *PIDE Blog*. https://pide.org.pk/blog/milk-production-in-pakistan/
- *Bovine—An overview | ScienceDirect Topics*. (n.d.). Retrieved August 18, 2024, from https://www.sciencedirect.com/topics/veterinary-science-and-veterinary-medicine/bovine
- Cháfer-Pericás, C., Maquieira, Á., & Puchades, R. (2010). Fast screening methods to detect antibiotic residues in food samples. *TrAC Trends in Analytical Chemistry*, *29*(9), 1038–1049. https://doi.org/10.1016/j.trac.2010.06.004

- Chen, J., Ying, G.-G., & Deng, W.-J. (2019). Antibiotic Residues in Food: Extraction, Analysis, and Human Health Concerns. *Journal of Agricultural and Food Chemistry*, *67*(27), 7569–7586. https://doi.org/10.1021/acs.jafc.9b01334
- *Dairy-2.pdf*. (n.d.). Retrieved July 7, 2024, from https://tdap.gov.pk/wp-content/uploads/2023/06/Dairy-2.pdf
- *Dror, D.K. and Allen, L.H. (2011) The Importance of Milk and Other Animal-Source Foods for Children in Low-Income Countries. Food and Nutrition Bulletin, 32, 227-243. - References—Scientific Research Publishing*. (n.d.). Retrieved August 18, 2024, from https://www.scirp.org/reference/referencespapers?referenceid=1382274
- *Frontiers | Prudent Use of Antibiotics in Dairy Cows: The Nordic Approach to Udder Health*. (n.d.). Retrieved August 18, 2024, from https://www.frontiersin.org/journals/veterinary-science/articles/10.3389/fvets.2021.623998/full
- Ghimpețeanu, O. M., Pogurschi, E. N., Popa, D. C., Dragomir, N., Drăgotoiu, T., Mihai, O. D., & Petcu, C. D. (2022). Antibiotic Use in Livestock and Residues in Food—A Public Health Threat: A Review. *Foods*, *11*(10), 1430. https://doi.org/10.3390/foods11101430
- Grau, G. (2017). Low-cost fabrication of paper-based systems: Microfluidics, sensors, electronics and deployment. *2017 IEEE 60th International Midwest Symposium on Circuits and Systems (MWSCAS)*, 84–87. https://doi.org/10.1109/MWSCAS.2017.8052866
- Guinati, B. G. S., Sousa, L. R., Oliveira, K. A., & Coltro, W. K. T. (2021). Simultaneous analysis of multiple adulterants in milk using microfluidic paper-based analytical devices. *Analytical Methods*, *13*(44), 5383–5390. https://doi.org/10.1039/D1AY01339D
- Kumar Rayappa, M., S, K. K., Rattu, G., & Murali Krishna, P. (2023). Advances and effectiveness of metal–organic framework-based bio/chemical sensors for rapid and ultrasensitive probing of antibiotic residues in foods. *Sustainable Food Technology*, *1*(2), 152–184. https://doi.org/10.1039/D2FB00035K

- Lu, Y., Shi, W., Qin, J., & Lin, B. (2010). Fabrication and characterization of paper-based microfluidics prepared in nitrocellulose membrane by wax printing. *Analytical Chemistry*, *82*(1), 329–335. https://doi.org/10.1021/ac9020193
- Martinez, A. W., Phillips, S. T., Whitesides, G. M., & Carrilho, E. (2010). Diagnostics for the developing world: Microfluidic paper-based analytical devices. *Analytical Chemistry*, *82*(1), 3–10. https://doi.org/10.1021/ac9013989
- Menkem, E., Ngangom, B., Tamunjoh, S., & Boyom, F. (2018). Antibiotic residues in food animals: Public health concern. *Acta Ecologica Sinica*, *39*. https://doi.org/10.1016/j.chnaes.2018.10.004
- Mesgari Abbasi, M., Babaei, H., Ansarin, M., Nourdadgar, A., & Nemati, M. (2011). Simultaneous Determination of Tetracyclines Residues in Bovine Milk Samples by Solid Phase Extraction and HPLC-FL Method. *Advanced Pharmaceutical Bulletin*, *1*(1), 34–39. https://doi.org/10.5681/apb.2011.005
- Nehra, M., Kumar, R., Dilbaghi, N., & Kumar, S. (2023). Controlled synthesis of Cu-MOF possessing peroxidase-mimetic activity for the colorimetric detection of tetracycline in aqueous solution. *New Journal of Chemistry*, *47*(16), 7595–7603. https://doi.org/10.1039/D3NJ00218G
- Nehra, M., Madan, R., Dilbaghi, N., & Kumar, S. (2023). Controlled synthesis of Cu-MOF possessing high peroxidase-mimetic activity for colorimetric detection of tetracycline in aqueous solution. *New Journal of Chemistry*, *47*. https://doi.org/10.1039/D3NJ00218G
- Patari, S., Datta, P., & Mahapatra, P. S. (2022). 3D Paper-based milk adulteration detection device. *Scientific Reports*, *12*(1), 13657. https://doi.org/10.1038/s41598-022-17851-3
- Rayappa, M., S., K., Rattu, G., & Krishna, P. (2023). Advances and effectiveness of metal–organic framework based bio/chemical sensors for rapid and ultrasensitive probing of antibiotic residues in foods. *Sustainable Food Technology*, *1*. https://doi.org/10.1039/D2FB00035K
- Sachi, S., Ferdous, J., Sikder, M. H., & Azizul Karim Hussani, S. M. (2019). Antibiotic residues in milk: Past, present, and future. *Journal of Advanced Veterinary and Animal Research*, *6*(3), 315–332. https://doi.org/10.5455/javar.2019.f350

- Seymour, E. H., Jones, G. M., & McGilliard, M. L. (1988). Persistence of Residues in Milk Following Antibiotic Treatment of Dairy Cattle. *Journal of Dairy Science*, *71*(8), 2292–2296. https://doi.org/10.3168/jds.S0022-0302(88)79806-9
- Souhila, A. H., Hamdi, B., & Brendlé, J. (2021). Tetracycline Removal from Water by Adsorption on Geomaterial, Activated Carbon and Clay Adsorbents. *Ecological Chemistry and Engineering S*, *28*, 303–328. https://doi.org/10.2478/eces-2021-0021
- Taghizadeh-Behbahani, M., Shamsipur, M., & Hemmateenejad, B. (2022). Detection and discrimination of antibiotics in food samples using a microfluidic paper-based optical tongue. *Talanta*, *241*, 123242. https://doi.org/10.1016/j.talanta.2022.123242
- *The State of Food and Agriculture 2019 | FAO*. (n.d.). Retrieved August 18, 2024, from https://www.fao.org/family-farming/detail/en/c/1245425/
- Vercelli, C., Amadori, M., Gambino, G., & Re, G. (2023). A review on the most frequently used methods to detect antibiotic residues in bovine raw milk. *International Dairy Journal*, *144*, 105695. https://doi.org/10.1016/j.idairyj.2023.105695
- *Veterinary Drug Detail | CODEXALIMENTARIUS FAO-WHO*. (n.d.). Retrieved August 18, 2024, from https://www.fao.org/fao-who-codexalimentarius/codex-texts/dbs/vetdrugs/veterinary-drug-detail/en/?d_id=12
- Younas, M., Maryam, A., Khan, M., Nawaz, A. A., Jaffery, S. H. I., Anwar, M. N., & Ali, L. (2019). Parametric analysis of wax printing technique for fabricating microfluidic paper-based analytic devices (µPAD) for milk adulteration analysis. *Microfluidics and Nanofluidics*, *23*(3). https://doi.org/10.1007/s10404-019-2208-z
- Yusuf, V. F., Malek, N. I., & Kailasa, S. K. (2022). Review on Metal–Organic Framework Classification, Synthetic Approaches, and Influencing Factors: Applications in Energy, Drug Delivery, and Wastewater Treatment. *ACS Omega*, *7*(49), 44507–44531. https://doi.org/10.1021/acsomega.2c05310

- Zhang, Q., Yan, S., Yan, X., & Lv, Y. (2023). Recent advances in metal-organic frameworks: Synthesis, application and toxicity. *Science of The Total Environment*, *902*, 165944. https://doi.org/10.1016/j.scitotenv.2023.165944
- Zheng, J., Zhang, J., Gao, L., Kong, F., Shen, G., Wang, R., Gao, J., & Zhang, J. (2020). The Effects of Tetracycline Residues on the Microbial Community Structure of Tobacco Soil in Pot Experiment. *Scientific Reports*, *10*. https://doi.org/10.1038/s41598-020-65203-w

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