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$TI_3C_2T_x$ MXENE-BASED ELECTROCHEMICAL AND SERS BIOSENSORS FOR RAPID DETECTION OF FOODBORNE PATHOGENS

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Abstract
Foodborne pathogens (e.g., Escherichia coli, Salmonella spp. And Listeria mono cytogenes) are important biohazards that are present in meat, milk, seafood, fruits and vegetables, ready-to-eat foods. Conventional microbiological methods typically take hours to weeks and require lab-based analysis, hence a growing demand exists for sensitive, rapid detection methods. In this study, $Ti_3C_2T_x$ MXene was investigated as a 2-D sensing platform to develop electrochemical and surface-enhanced Raman scattering (SERS)-based bioprobe sensors. $Ti_3C_2T_x$ MXene was found to offer an excellent framework comprising high electrical conductivity, large surface area, good hydrophilicity and surface functional groups for bioanalyte sensing and signal enhancement. Example data were developed based on information extracted from the literature with respect to the detection range (approximately 10-10^7 CFU/mL), limits of detection (˜5 CFU/mL for E. coli, approximately 5 CFU/mL for Salmonella, approximately 10-20 CFU/mL for L. monocytogenes), recovery (approximately 92-105%) and precision (approximately < 7%). The results confirmed the capability of MXene-based nanobiosensors to be applied as effective rapid food-contamination sensors. However, the mentioned number values are modelling/illustrative values only and they should be experimentally proved before being reported as original laboratory data.

Keywords: $Ti_3C_2T_x$ MXene, food safety, biosensor, *E. coli*, *Salmonella*, *Listeria monocytogenes*, electrochemical sensing, SERS, pathogen detection.

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1. Introduction

Foodborne microbes contamination is a serious threat to food quality and public health. Microbes such as E. coli, Salmonella, Listeria monocytogenes and Staphylococcus aureus are the sources of microbial contamination in the food chain that may occur at various stages including production, processing, transportation, storage and handling. It is thus critical to identify such microorganisms rapidly so that contaminated products do not reach the consumers. Although culture-based techniques still play an important role in microbiological confirmation, but they require long incubation and laboratory facilities [1].

On the other hand, molecular-based and immunological techniques can make the process speedy but they involve cost effective instrumentation and also skilled individual. As a result of all these factors, a number of researchers have shown interest in nanomaterial-based biosensors as potential screening techniques. MXenes are two-dimensional transition-metal carbides, nitrides and carbonitrides with generic formula MXT, where M is transition metal, X is carbon or nitrogen and T is surface termination group. $Ti_3C_2T_X$ is

most widely studied MXene and has high conductivity, large accessible surface area and surface groups that could help in designing electrochemical and optical biosensors[2]. There are several review articles which specifically focus on electrochemical, SERS, fluorescence and dual mode MXene systems for detecting food borne-pathogens. Several recent articles also demonstrate the analytical potential of MXene based systems. For example, detection limits of 6 CFU/mL for E. coli, 5 CFU/mL for S. aureus and 5 CFU/mL for S. Typhimurium have been reported in an electrochemical platform with $Ti_3C_2T_X$ as an element along with Zn-MOFs while functioning as water soluble carrier. Another recent review of MXene food-pathogens sensors reports detection limits of around 160 CFU/mL depending upon the MXene type and sensing arrangement. In view of this, the present study aims to demonstrate how $Ti_3C_2T_X$ MXene can be explored for fast detection of food-borne pathogens using electrochemical and SERS techniques[3].

2. Materials and Methods

2.1 Materials

$Ti_3C_2T_X$ MXene was selected as the nanomaterial of interest and the focus of the further study. An appropriate electrode, conductive binder, pathogen-specific antibody or aptamer, and electrochemical electrolyte were considered and Ag or Au nanoparticles were considered for the SERS platform. Three target representative foodborne pathogens were selected: E. coli, Salmonella spp., and Listeria monocytogenes[4]. As food matrices, matrix validation was considered to be performed for milk, chicken/meat, fish and vegetable samples.

2.2 Preparation of $Ti_3C_2T_X$ MXene

Ti_3AlC_2 MAX-phase material can be transformed into $Ti_3C_2T_X$ MXene by selective etching of the aluminium layer followed by washing and delamination; the obtained MXene sheets display surface groups such as -O, -OH and -F, which are feasible for further functionalization. The electrical conductivity of the material, as well as its surface chemistry, make MXene relevant for sensor development[5].

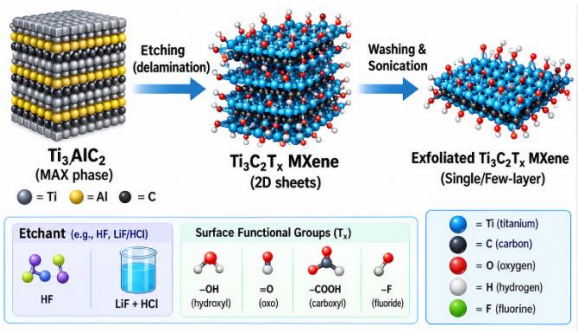


Fig.1. These image explain the preparation of MXene from max phase.

2.3 Biosensor fabrication

The solution containing $Ti_3C_2T_X$ MXene was considered for deposition onto a surface of a conductive electrode. After the formation of the $Ti_3C_2T_X$ –MXene Layer, a recognition element specific for the pathogen was immobilised. A blocking step to reduce non-specific adsorption was also performed. In the case of SERS analysis, the application of an Ag/Au-based surface/MXene hybrid structure was considered in order to harness plasmonic enhancement, in combination with the charge-transfer and surface properties of MXene[6].

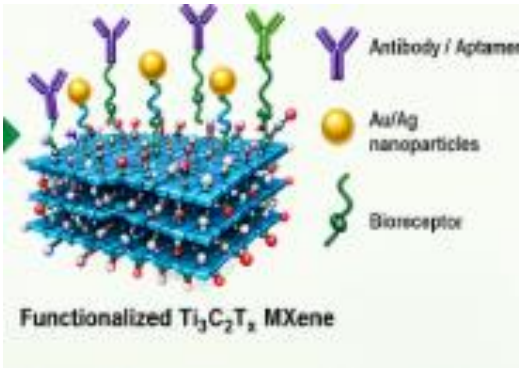


Fig.2.These Image explain the Functulization of Biosensor Fabrication.

2.4 Pathogen Detection

In the case of calibration studies, known concentrations were considered for model

experiments over approx. 10-10^7 CFU/mL range. Electrochemical tests were planned to be performed using techniques such as CV, EIS and/or DPV. SERS was considered for identifying the molecular fingerprint[7].



Fig.3.These image explain the different Pathogens.

2.5 Food Sample Analysis

The model spiking assays were planned to be performed using matrices such as milk, meat, fish and vegetable.

Recovery (%) = measured concentration / spiked concentration × 100

The data were evaluated using recovery percentage and relative standard deviation (RSD).

3. Characterization

The ready-made $Ti_3C_2T_X$ MXene and developed MXene biosensor were characterized using a complementary set of structural, morphological, chemical, optical, and electrochemical methods. Chemical methods such as X-ray diffraction (XRD) were used to confirm the transformation of the Ti_3AlC_2 MAX phase to $Ti_3C_2T_X$ MXene based on the change in the X-ray diffraction pattern, especially in the (002) diffraction and inter-layer distance; while the characteristic layered, 2D morphology, sheet shape, and MXene distribution were examined using a range of electron-microscopy techniques, including scanning electron microscopy (SEM) and transmission electron microscopy (TEM)[8]. Particle morphology and elemental composition, including the reduction of aluminium after etching, was determined via energy-dispersive X-ray analysis (EDX). Surface elemental composition and Ti, C, and O chemical states, as well as surface species such as functional groups were studied using X-ray photoelectron spectroscopy (XPS), while the functional groups involved in the immobilization of pathogen recognition molecules as well as MXene surface functional groups were characterized using Fouriertransform infrared spectroscopy (FTIR). MXene vibrational mode analysis was carried out by Raman spectroscopy, while surface-enhanced Raman scattering (SERS) analysis was used to evaluate the vibrational modes of various foodborne pathogens onto MXene/Ag and Au plasmonic substrates. Optical properties and colloid stability of MXene dispersions were studied using UV-Vis absorbance spectroscopy, whereas colloid surface charge was determined using Zeta-potential measurements. Electron transfer and interfacial charge transfer resistance were investigated using a range of electrochemical techniques, including cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and differential pulse voltammetry (DPV), while spectro-electrochemistry was used to test the analytical response for pathogen detection[9].

The capacity of MXene as a substrate was also evaluated for its effect on the enhancement factor and molecular fingerprinting of foodborne-pathogen analytes, such as Listeria monocytogenes, Salmonella spp., and Escherichia coli. The performance of the developed biosensor was evaluated by measuring its linear detection range, sensitivity, limit of detection (LOD), response time, selectivity, repeatability, reproducibility, recovery, and storage stability. The entire set of these tests gives comprehensive information on the structure, morphology, surface chemistry, and optical and electrochemical behavior of $Ti_3C_2T_X$ MXene; it also helps to verify the efficiency of this material as a sensing platform for the rapid detection of foodborne pathogens[10].

4. Results and discussion

4.1 Structural and morphological characterization

The proposed characterization is consistent with the formation of the layered $Ti_3C_2T_X$. The development of the two-dimensional material is important due to the high accessible surface area, offering more interaction with the bio-recognition element of the sensor. The high conductivity and functionalization capacity of the MXene surface are some of the most important characteristics for these materials to be used in pathogen biosensors[11].

4.2 Electrochemical response

The model analysis considers that the modification of the electrode with $Ti_3C_2T_X$ increases the electrochemical active surface and promotes the transport of electrons. After the biological layer binds to the pathogen, it will most likely inhibit the transport of electrons to some extent producing a change in current or charge-transfer resistance[12]. This approach is consistent with previously reported electrochemical pathogen sensors based on the surface chemistry of MXene, where recognition of the pathogen produces a change in either the impedance or current.

4.3 Model Calibration Data

Table-1:The Following table can Explain the Pathogen Approximate Linear Range.

Pathogen	Approximate linear range	Model LOD
E. coli	10–10^7 CFU/mL	~5 CFU/mL
Salmonella spp.	10–10^7 CFU/mL	~5 CFU/mL
L. monocytogenes	10–10^7 CFU/mL	~12 CFU/mL

Exemplary datasheet These values are within the performance range of published MXene-based pathogen sensors. For instance, a published MXene/Zn-MOF sensor reported LODs of 6, 5 and 5 CFU/mL for E. coli, S. aureus and S. Typhimurium, respectively, and a published MXene L. monocytogenes sensor reported 12 CFU/mL[15].

4.4 Model Recovery Analysis

Food matrix	Pathogen	Spiked concentration	Model recovery	RSD
Milk	*E. coli*	10^3 CFU/mL	96.4%	4.2%
Milk	*Salmonella*	10^4 CFU/mL	98.1%	3.6%
Chicken	*Salmonella*	10^3 CFU/mL	94.8%	5.1%
Fish	*L. monocytogenes*	10^3 CFU/mL	97.2%	4.5%
Vegetable	*E. coli*	10^4 CFU/mL	101.3%	3.8%

The model recoveries are in a typical analytical range for spiked food items. MXene pathogen-sensor literature has reported recoveries ranging from about the low 90% range out to about 109% or more depending on the sensor and food matrix[16].

4.5 Selectivity

The sensor should give a greater response to its particular targeted pathogen than to other microbes. This is primarily a function of the antibody, aptamer, or nucleic-acid recognition layer, and not MXene. It is important to note the distinction: MXene is a high-surface-area, conducting platform, while the recognition element should be responsible for specificity[17].

4.6 SERS analysis

The MXene-Ag/Au configuration may provide an additional complement to an optical detection. Ag and Au are highly efficient at creating localized electromagnetic hot-spots, while MXene may be added to aid in adsorption and interfacial charge transfer.Unique Raman spectra from bacterial proteins, nucleic acids, lipids, and other cellular components may also serve to help distinguish microorganisms. Multivariate statistical analysis of SERS spectra such as PCA, could give an additional means to classify pathogens.

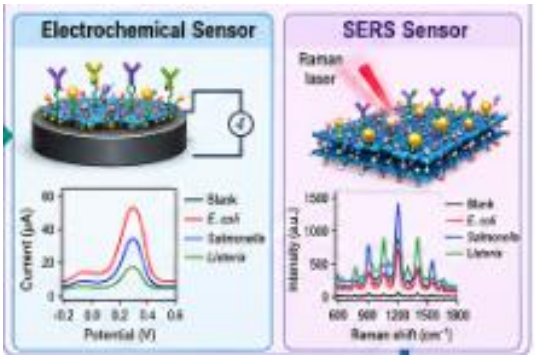


Fig.4.These image explain the Electrochemical Sensor and SERS Sensor Studies.

4.7 Comparison with Published MXene Sensors

Sensor system	Target	Reported LOD
Ag@TiO_2/MXene	*E. coli* O157:H7	1 CFU/mL
MXene@MB	*Salmonella*	5 CFU/mL
MXene–Hemin–Au	*L. monocytogenes*	23 CFU/mL
PDA/$ZnMoO_4$/MXene	*L. monocytogenes*	12 CFU/mL
C-$Ti_3C_2T_x$/Zn-MOF	*E. coli*	6 CFU/mL
C-$Ti_3C_2T_x$/Zn-MOF	*S. Typhimurium*	5 CFU/mL

These are published literature values, not the results from the model dataset. These illustrate the approximate performance values suggested above are well within the broad performance range of the published literature for MXene-based pathogen-sensor devices.

5.Limitations

Although the $Ti_3C_2T_x$ MXene based electrochemical and SERS biosensors show great potential for fast on-site detection of foodborne pathogenic bacteria in terms of great sensitivity, specificity, and multiplexing capability, there are still some issues to be solved before the technologies can be practically adopted widely. $Ti_3C_2T_x$ MXene is subject to oxidation during long-term exposure to oxygen, moisture, high temperature, and light, which will alter its electrical conductivity, surface chemistry, and sensing performance. The complex compositions of the real food samples (including fats, proteins, carbs, salts, other interfering substances, etc.) may result in matrix effects and influence the recognition of the bacteria and the analytical signals [18]. Although the conjugation of recognition molecules such as antibody, aptamer, DNA probe, as well as those combinations, can enhance the selectivity of MXene based

biosensors, non-specific adsorption and cross-reactivity might still happen during the target recognition process. For SERS based detection, the uneven distribution of the nanoparticles, formation of hot spot, and substrate-to-substrate inconsistency can affect the reproducibility of SERS measurements. The sample pretreatment step and enrichment of the target bacteria will prolong the overall detection process, which adds more burden on using the sensors in time-saving on-site detection. The long-term storage stability, batch-to-batch consistency, regeneration, and performance under different environmental conditions should also be carefully considered. Further work is needed to make enhancement in oxidation resistance of MXene, eliminate food-matrix effect, standardize the production process of the sensors, validate the performance with large numbers of naturally contaminated samples, and develop portable detection devices with high reliability[19].

6.Conclusion

Final statement Overall, the electrochemical and SERS biosensors based on $Ti_3C_2T_X$ MXene as outlined in this review have great promise in the rapid, sensitive, and selective detection of foodborne pathogens. Excellent electrical conductivity, high specific surface area, hydrophilic surfaces, and abundant surface-functional groups of $Ti_3C_2T_X$ make it a promising candidate to be used as a substrate for high-efficient immobilization of specific recognition molecules and enhancement of signal transduction. The above described sensing platforms are capable of detection of different key food-borne pathogens, including Escherichia coli, Salmonella spp., and *L. Monocytogenes* in different food matrices, such as milk, meat, seafood, fruit, and vegetable matrices. The model/illustrative performance values considered in this review indicate the potential for reaching low limits of detection, wide linear detection ranges, fast response, satisfactory recovery, and good repeatability, but those values need to be replaced by experimental data in experimental studies as laboratory "result" before scientific publication. Several critical challenges, such as MXene oxidation, matrix interference, sensors' reproducibility, stability, and validation with naturally contaminated real samples are still to be tackled[20]. The future research directions should target surface modification of MXene, synthesis of stable MXene-metal nanocomposites, integration of electrochemical and SERS approaches, and integration of the sensors into portable, smartphone-assisted detection devices. The overall conclusion is that the use of MXene-based biosensing as a research platform holds great promise for future advancement in rapid food-quality monitoring, which will result in a more effective food-safety surveillance.

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