

**DEVELOPMENT OF NOVEL NANOSTRUCTURES  
AND NANOCOMPOSITES FOR MELAMINE  
DETECTION**

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AND NANOCOMPOSITES FOR MELAMINE  
DETECTION**

*By*

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*Submitted*

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to the**



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## **Certificate**

This is to certify that the thesis entitled "**Development of novel nanostructures and nanocomposites for melamine detection,**" submitted by **Mr. Mohit Tiwari** to the Indian Institute of Technology Delhi for the award of the degree of Doctor of Philosophy, is a record of the original bonafide research work carried out by him. He has worked under my supervision and has fulfilled the requirements, which to my knowledge, has reached the requisite standard for the submission of this thesis. The results in this thesis have not been submitted in part or whole to any University or institute to award any degree or diploma.

**Prof. Sudip K. Pattanayek**  
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**Mohit Tiwari**

## **Abstract**

Melamine is a substance that can be found in a variety of foods. Melamine is a nitrogen-rich compound that has garnered significant concern due to widely publicized food safety incidents. Melamine has been synthetically and fraudulently utilized as an unnatural nitrogen ingredient in a variety of food items in an attempt to enhance the "false" apparent protein composition of these products.

Various traditional techniques are available for detecting Melamine, but these techniques require sophisticated instruments, a technical workforce, and significant capital investments. To overcome these issues, researchers have studied different sensors that may be more efficient for on-site and specific detection of melamine. Even though there has been much study on the topic, a sensor platform with high sensitivity and selectivity is still unavailable. Thus, it is essential to have a sensor substrate for the accurate and specific monitoring of melamine traces. Thorough knowledge of melamine interactions with appropriate sensing molecules is essential to develop such a sensing platform.

The purpose of this research was to examine the potential of surface-enhanced Raman spectroscopy (SERS) and novel molecularly imprinted polymers (MIPs) for detecting Melamine in a typical dairy product (i.e., milk). Additionally, this effort focuses on developing innovative nanostructured materials for use in sensor manufacturing to detect Melamine through several techniques, such as vacuum reflux, photoreduction, and sol-gel.

Surface-enhanced Raman scattering (SERS) has attracted considerable attention in biological and chemical identification as a simplified, quick, and ultrasensitive analysis method. We have selected a case of oxide functional oxide organic nanostructure between  $\text{ZnFe}_2\text{O}_4$  and  $\text{ZnO}$ , denoted as ZZF. The material was used to identify Melamine in the concentration range of  $0.39 \mu\text{M}$ – $7.92 \mu\text{M}$ . This high-performance nanocomposite provides improved melamine sensitivity toward SERS, and the detection limit is as low as  $0.39 \mu\text{M}$ .

The performance of the SERS substrate was further enhanced by preparing a  $\text{g-C}_3\text{N}_4/\text{Ni}_3\text{N}$  ( $\text{Au/g-C}_3\text{N}_4/\text{Ni}_3\text{N}$ ) nanocomposite material. The intended nanocomposites were synthesized using a simple and cost-effective fabrication process. The  $\text{Au/g-C}_3\text{N}_4/\text{Ni}_3\text{N}$  was synthesized by photo-reducing  $\text{HAuCl}_4$ . The developed SERS-based nanocomposite can detect R6G with an enhancement factor of  $1.82 \times 10^8$ , whereas, in the case of Melamine, the average enhancement factor is evaluated to be  $2.56 \times 10^7$ .

There is a significant challenge in detecting and eliminating Melamine from low concentrations solutions. A specific site is needed to interact with and adsorb a specified quantity of Melamine.

Understanding the adsorption process in detail is essential for fabricating substrates that result in the adsorption of Melamine. Here, we investigated the effects of Melamine on surfaces that had been functionalized with one of four silane coupling agents: n-propyltrimethoxy silane (PTMS), octyltrimethoxy silane (OTMS), 3-(trimethoxysilyl) propyl methacrylate (TMSPMA), or 3-(triethoxysilyl) propionitrile (TESPN). Real-time QCM-D adsorption data for Melamine over a number of substrates are used to fit the model. According to the findings, melamine molecules are adsorbing more effectively to the TMSPMA functionalized surface since it is the most hydrophilic of the tested surfaces. There is good agreement between the theoretically calculated mass for a TMSPMA-functionalized silica surface and the experimentally measured mass of Melamine adsorbed in a monolayer. Adsorption is understood to take place because of interactions between molecules. Since these surface functions can interact with multiple molecules with the same terminal groups, they do not give selectivity to a single molecule. The selectivity of the Quartz crystal microbalance (QCM) sensor was improved using a molecularly imprinted polymer (MIP). We employed the sol-gel method to manufacture the MIP with 3-(trimethoxysilyl) propyl methacrylate (TMSPMA) as the functional monomer. In order to detect Melamine in a QCM-D silica-quartz crystal, the well MIP was employed. Melamine molecules bind strongly to MIP surfaces in real-time sorption experiments when utilized as reference molecules. The developed MIP is significantly specific for Melamine in investigations testing both specificity and reproducibility. This research sheds light on the fundamental interaction capabilities of Melamine with a suitable substrate, which can be converted into a user-friendly selective sensor for their detection and elimination.

## सार

मेलामाइन एक ऐसा पदार्थ है जो विभिन्न प्रकार के खाद्य पदार्थों में पाया जा सकता है। मेलामाइन एक नाइट्रोजन युक्त यौगिक है जो व्यापक रूप से प्रचारित खाद्य सुरक्षा घटनाओं के कारण महत्वपूर्ण चिंता का विषय है। इन उत्पादों की "झूठी" स्पष्ट प्रोटीन संरचना को बढ़ाने के प्रयास में विभिन्न खाद्य पदार्थों में मेलामाइन को कृत्रिम रूप से और धोखाधड़ी से गैर-प्रोटीन नाइट्रोजन घटक के रूप में उपयोग किया गया है।

मेलामाइन का पता लगाने के लिए विभिन्न पारंपरिक तकनीकें उपलब्ध हैं, लेकिन इन तकनीकों के लिए परिष्कृत उपकरणों, एक तकनीकी कार्यबल और महत्वपूर्ण पूंजी निवेश की आवश्यकता होती है। इन मुद्दों को दूर करने के लिए, शोधकर्ताओं ने विभिन्न सेंसरों का अध्ययन किया है जो साइट पर और मेलामाइन की विशिष्ट पहचान के लिए अधिक कुशल हो सकते हैं। भले ही इस विषय पर बहुत अध्ययन किया गया हो, उच्च संवेदनशीलता और चयनात्मकता वाला एक सेंसर प्लेटफॉर्म अभी भी अनुपलब्ध है। इस प्रकार, मेलामाइन के निशान की सटीक और विशिष्ट निगरानी के लिए एक सेंसर सबस्ट्रेट होना आवश्यक है। इस तरह के सेंसिंग प्लेटफॉर्म को विकसित करने के लिए उपयुक्त सेंसिंग अणुओं के साथ मेलामाइन की बातचीत का गहन ज्ञान आवश्यक है।

इस शोध का उद्देश्य एक विशिष्ट डेयरी उत्पाद (यानी दूध) में मेलामाइन का पता लगाने के लिए सतह-संवर्धित रमन स्पेक्ट्रोस्कोपी (एसईआरएस) और उपन्यास आणविक रूप से अंकित पॉलिमर (एमआईपी) की क्षमता की जांच करना था। इसके अतिरिक्त, यह प्रयास कई तकनीकों, जैसे वैक्यूम रिफ्लेक्स तकनीक, फोटोरिडक्शन और सोल-जेल के माध्यम से मेलामाइन का पता लगाने के लिए सेंसर निर्माण में उपयोग के लिए नवीन अति सूक्ष्म संरचना सामग्री विकसित करने पर केंद्रित है।

सतह-संवर्धित रमन स्पेक्ट्रोस्कोपी ने एक सरल, त्वरित और अल्ट्रासेंसिटिव विश्लेषण पद्धति के रूप में जैविक और रासायनिक पहचान में काफी ध्यान आकर्षित किया है। हमने जिंक फेराइट ( $ZnFe_2O_4$ ) और जिंक ऑक्साइड ( $ZnO$ ) के बीच ऑक्साइड कार्यात्मक ऑक्साइड कार्बनिक नैनोस्ट्रक्चर के एक मामले का चयन किया है, जिसे ZZF के रूप में दर्शाया गया है। सामग्री का उपयोग  $0.39 \mu M$ – $7.92 \mu M$  की एकाग्रता सीमा में मेलामाइन की पहचान करने के लिए किया गया था। यह उच्च-प्रदर्शन अति सूक्ष्म संमिश्रित पदार्थ एसईआरएस के प्रति बेहतर मेलामाइन संवेदनशीलता प्रदान करता है, और पता लगाने की सीमा  $0.39 \mu M$  जितनी कम है।

$g-C_3N_4/Ni_3N$  अति सूक्ष्म संमिश्रित पदार्थ तैयार करके सतह वर्धित रमन प्रकीर्णन अंतर्निहित परत के प्रदर्शन को और बढ़ाया गया। एक सरल और लागत प्रभावी निर्माण प्रक्रिया का उपयोग करके इच्छित नैनोकम्पोजिट को संश्लेषित किया गया था।  $Au/g-C_3N_4/Ni_3N$  को प्रकाशिक अपचयन करने वाले क्लोरोऑरिक एसिड ( $HAuCl_4$ ) द्वारा संश्लेषित किया गया था। विकसित सतह वर्धित रमन प्रकीर्णन -आधारित अति सूक्ष्म संमिश्रित पदार्थ  $1.82 \times 10^8$  के वृद्धि कारक के साथ

रोडामाइन ६जी का पता लगा सकता है, जबकि  $c$  के मामले में, औसत वृद्धि कारक का मूल्यांकन  $2.56 \times 10^7$  किया जाता है।

कम सांद्रता वाले समाधानों से मेलामाइन का पता लगाने और उसे खत्म करने में एक महत्वपूर्ण चुनौती है। मेलामाइन की एक निर्दिष्ट मात्रा के साथ बातचीत करने और सोखने के लिए एक विशिष्ट साइट की आवश्यकता होती है। सोखना प्रक्रिया को विस्तार से समझना उन सबस्ट्रेट्स के निर्माण के लिए आवश्यक है जिसके परिणामस्वरूप मेलामाइन का सोखना होता है। यहां, हमने उन सतहों पर मेलामाइन के प्रभावों की जांच की जिन्हें चार सिलेन युग्मन एजेंटों में से एक के साथ क्रियाशील किया गया था: एन-प्रोपाइलट्रिमेथॉक्सी सिलाने (पीटीएमएस), ऑक्टाइलट्रिमेथॉक्सी सिलाने (ओटीएमएस), 3-(ट्राइमेथॉक्सीसिलिल) प्रोपाइल मेथैक्रिलेट (टीएमएसपीएमए), या 3-(ट्राइथॉक्सीसिलिल) प्रोपियोनिट्राइल (टीईएसपीएन)। कई सबस्ट्रेट्स पर मेलामाइन के लिए रीयल-टाइम क्यूसीएम-डी सोखना डेटा मॉडल को फिट करने के लिए उपयोग किया जाता है। निष्कर्षों के अनुसार, मेलामाइन अणु टीएमएसपीएमए कार्यात्मक सतह पर अधिक प्रभावी ढंग से सोख रहे हैं क्योंकि यह परीक्षण की गई सतहों का सबसे हाइड्रोफिलिक है। एक टीएमएसपीएमए -कार्यात्मक सिलिका सतह के लिए सैद्धांतिक रूप से गणना किए गए द्रव्यमान और एक मोनोलेयर में अधिशोषित अधिशोषित के प्रयोगात्मक रूप से मापा द्रव्यमान के बीच अच्छा समझौता है। यह समझा जाता है कि अणुओं के बीच परस्पर क्रिया के कारण सोखना होता है। चूंकि ये सतह कार्य एक ही टर्मिनल समूहों के साथ कई अणुओं के साथ बातचीत कर सकते हैं, वे एक अणु को चयनात्मकता नहीं देते हैं। आणविक रूप से अंकित बहुलक (एमआईपी) का उपयोग करके क्वार्ट्ज क्रिस्टल माइक्रोबैलेंस (क्यूसीएम) सेंसर की चयनात्मकता में सुधार किया गया था। हमने एमआईपी के निर्माण के लिए सोल-जेल विधि को 3-(ट्राइमेथोक्सीसिलिल) प्रोपाइल मेथैक्रिलेट (टीएमएसपीएमए) के साथ कार्यात्मक मोनोमर के रूप में नियोजित किया। क्यूसीएम-डी सिलिका-क्वार्ट्ज क्रिस्टल में मेलामाइन का पता लगाने के लिए, कुएं एमआईपी को नियोजित किया गया था। संदर्भ अणुओं के रूप में उपयोग किए जाने पर मेलामाइन अणु वास्तविक समय के सोखने के प्रयोगों में एमआईपी सतहों से मजबूती से जुड़ते हैं। विकसित एमआईपी विशिष्टता और पुनरुत्पादकता दोनों की जांच में मेलामाइन के लिए काफी विशिष्ट है। यह शोध एक उपयुक्त सबस्ट्रेट के साथ मेलामाइन की मौलिक अंतःक्रियात्मक क्षमताओं पर प्रकाश डालता है, जिसे उनकी पहचान और उन्मूलन के लिए उपयोगकर्ता के अनुकूल चयनात्मक सेंसर में परिवर्तित किया जा सकता है।

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**List of Abbreviations**

AFM	Atomic Force Microscopy
AuNP	Gold Nanoparticles
CA	Contact angle
CB	Conduction Band
DFT	Density Functional Theory
EDX	Energy Dispersive X-ray (spectroscopy)
FESEM	Field Emission Scanning Electron Microscopy
FTIR	Fourier Transformed Infra-red (spectroscopy)
GC	Gas Chromatography
gC <sub>3</sub> N <sub>4</sub>	Graphitic Carbon Nitride
HPLC	High-Performance Liquid Chromatography
HRTEM	High-Resolution Transmission Electron Microscopy
MS	Mass spectrometry
Ni <sub>3</sub> N	Nickel Nitride
OTES	Triethoxy(octyl)silane
OTMS	Octyl trimethoxy silane
PTMS	n- propyl trimethoxy silane
QCM-D	Quartz crystal microbalance with dissipation
SAMs	Self-assembled monolayers
SEM	Scanning Electron Microscopy
SERS	Surface Enhanced Raman Scattering
TEM	Transmission Electron Microscopy
TESPN	3-(Triethoxysilyl) propionitrile
TMSPMA	3-(Trimethoxysilyl) propyl methacrylate
VB	Valence Band

## *List of Abbreviations*

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WHO	World Health Organization
XPS	X-ray Photoelectron Spectroscopy
XRD	X-ray Diffraction
ZnFe <sub>2</sub> O <sub>4</sub>	Zinc Ferrite
ZnO	Zinc Oxide