

**STUDIES ON METAL-CLAY COMPLEX BASED
HDPE NANOCOMPOSITES FOR BIOMEDICAL
APPLICATIONS**

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APPLICATIONS**

by
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Submitted

in fulfillment of the requirements of the degree of Doctor of Philosophy

to the



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To,

Wisdom, Peace and Happiness

CERTIFICATE

This is to certify that the thesis entitled “**STUDIES ON METAL-CLAY COMPLEX BASED HDPE NANOCOMPOSITES FOR BIOMEDICAL APPLICATIONS**” being submitted by **Ms. ANASUYA ROY** to Indian Institute of Technology Delhi for the award of the degree of “**DOCTOR OF PHILOSOPHY**” is a record of the authentic research work carried out by her under our supervision and guidance. She has fulfilled all the requirements for submission of this thesis, which to the best of our knowledge has reached the required standard.

The material contained in this thesis has not been submitted in part or full to any other University or Institute for the award of any other degree.

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Anasuya Roy

Abstract

A series of HDPE nanocomposites based on metal-clay complex with antimicrobial and cytocompatible properties were developed with an aim to reduce nosocomial infections acquired in hospitals and out-patient clinics. The properties of the nanocomposite are highly dependent on the type of synthesized metal-clay complex and its uniform dispersion in the matrix. The work of the present thesis is divided into 7 (seven) research chapters. The synthesis and properties of the metal-clay complex are covered in the first two chapters and properties and applications of the metal-clay in HDPE matrix in the next three chapters. The first chapter describes synthesis, characterization and antimicrobial properties of ion (Ag^+ , Cu^{2+} and Zn^{2+})-exchanged montmorillonite (M-MMT) synthesized from sodium montmorillonite (Na-MMT). The effect of synthesis parameters (acid activation and metal precursor concentration) on the loading of cations onto MMT is discussed. EDAX and ICP-OES confirmed successful loading of elemental Ag, Cu and Zn in all three types of cation exchanged MMT whereas WAXD analysis showed undisturbed crystal structure of MMT with increased interlayer space compared to Na-MMT. TEM micrographs revealed formation of spherical Ag nanocrystals in Ag-MMT on the supporting silicate layers although no external reducing agent was employed. However, nanoparticles (NPs) were not observed in case of Cu and Zn exchanged MMT. The bactericidal efficacy of the metal-clay complex was evaluated against Gram (-) *E. coli*, Gram (+) *S. aureus* and black mold fungi *A. niger* which showed large inhibition zones for all the three types of exchanged MMTs. The minimum inhibitory concentration (MIC) of the clay minerals were evaluated.

Nanoparticles (NPs) deposited over supports such as MMT have advantages over unsupported NPs in terms of stability, reactivity and release characteristics. The second chapter deals with study on synthesis and properties of Ag, Cu and ZnO NPs deposited over MMT (NP-

MMT). The NP decorated MMTs were synthesized from cation exchanged MMT (M-MMT) and the NP formation was facilitated using various chemical and physical reduction routes. The strength of the reducing medium was found to play a crucial role in deciding the seed/nuclei density and thereby determining the final NP size. Significantly higher loading of metallic elements was observed in NP-MMT clays by EDAX and ICP-OES analysis than M-MMT. The metal NP-MMT complex exhibited pronounced degree of antimicrobial activity against *E. coli*, *S. aureus* and *A. niger*. The ranking of antimicrobial activity in three types of cations in NP decorated MMT is similar to that of cation exchanged MMT.

The degree of dispersion of MMT platelets decides the nanocomposite morphology and is a key factor in governing its resultant properties. The third chapter deals with optimization of process parameters of twin screw compounding using a Design of Experiment (DoE) approach to achieve the best mechanical and antimicrobial behavior. In the fourth chapter, HDPE nanocomposites based on M-MMT and NP-MMT were developed by melt compounding route. The nanocomposites were prepared by varying synthesized MMT concentrations (1-5 wt%) and further processed to prepare filaments, films and molded specimens. The nanocomposites showed exfoliated clay morphology at lower concentrations and a mixture of intercalated and exfoliated structures at higher concentrations of clay as confirmed by TEM and WAXD analysis. Rheological characterization and crystallization studies on synthesized nanocomposites were also performed. The mechanical testing data revealed tensile strength and modulus improvement after incorporation of MMT with a marginal decrease in strain-at-break value.

In the fifth chapter, the antimicrobial and cytocompatible behavior of HDPE/M-MMT and HDPE/NP-MMT nanocomposites have been presented. A comparative analysis between antimicrobial activities of molded specimen, film and filament has been presented to study the

correlation of different specimen forms to their antimicrobial behavior. Filaments and films of HDPE/metal-clay nanocomposites showed higher activity than corresponding molded specimens due to higher diffusion rates owing to larger surface area exposure. Differences were also observed between M-MMT nanocomposites and NP-MMT nanocomposites. The quantity of freely available ions rather than its loading was observed to influence the extent of antimicrobial activity. Obviously, there were also significant differences in antimicrobial behaviour for three different types of metal ions/NPs. The mechanism of antimicrobial activity was studied from bacteria/NP interaction obtained from TEM micrographs. The release behaviour of free metal ions from M-MMT and NP-MMT was studied by ICP-MS. As expected, burst release is more prominent in corresponding M-MMT than NP-MMT. The durability of the antimicrobial activity of the nanocomposites was evaluated using time-kill assay under accelerated conditions.

To address the growing concerns for nano-toxicology and its impact on human health, the HDPE nanocomposites were evaluated against human cell lines *in vitro* to quantify the extent of toxicity on intimate contact with human skin and blood. RBC hemolysis protection assay was performed with human RBC cells and cytocompatibility was evaluated with human dermal fibroblast cells in MTT assay. The metal-clay complexes, both M-MMT and NP-MMT showed incompatibility to some extent, especially Ag based clays. However, complete compatibility was achieved in the highest loaded nanocomposite even after 48 h of continuous agitation. *In vivo* cytocompatibility testing was done on Sprague-Dawley rats surgically implanted with HDPE nanocomposite films. Histopathological microscopic analysis of the skin tissue sections retrieved from the exposed part was conducted post 21 days. No abnormalities were observed in epidermis indicating complete safety of the skin tissues exposed to HDPE/metal-clay complex nanocomposites.

सार

रोगाणुरोधी और साइटोकम्पैटिबल गुणों के साथ धातु-मिट्टी (Metal-clay) के परिसर पर आधारित HDPE नैनोकम्पोजिट की एक श्रृंखला को अस्पतालों और आउट-पेशेंट क्लीनिकों में अधिग्रहित नोसोकोमियल संक्रमण को कम करने के उद्देश्य से विकसित किया गया था। नैनोकम्पोसाइट के गुण संश्लेषित धातु-मिट्टी के परिसर और मैट्रिक्स में इसके एकसमान फैलाव पर अत्यधिक निर्भर हैं। वर्तमान थीसिस का काम 7 (सात) अनुसंधान अध्यायों में विभाजित है। धातु-मिट्टी कॉम्प्लेक्स का संश्लेषण और गुण पहले दो अध्यायों में शामिल हैं और अगले तीन अध्यायों में HDPE में धातु-मिट्टी के गुण और अनुप्रयोग शामिल हैं। पहले अध्याय में धातु-कण (Ag^+ , Cu^{2+} और Zn^{2+}) के संश्लेषण, लक्षण वर्णन और रोगाणुरोधी गुणों का वर्णन किया गया है - सोडियम मॉन्टमोरिलोनोइट (Na-MMT) से संश्लेषित मॉन्टमोरिलोनोइट (M-MMT)। MMT पर उद्धरण के लोड पर संश्लेषण मापदंडों (एसिड सक्रियण और धातु अग्रदूत एकाग्रता) के प्रभाव पर चर्चा की गई है। EDX और ICP-OES ने तीनों प्रकार के उद्धरण में MMT का आदान-प्रदान करने के लिए तत्व Ag, Cu और Zn के सफल लोडिंग की पुष्टि की, जबकि WAXD विश्लेषण ने MMT के अबाधित क्रिस्टल संरचना को Na-MMT की तुलना में वृद्धि हुई इंटरलेयर स्पेस के साथ दिखाया। TEM माइक्रोग्रैफ़्स ने सपोर्टिंग सिलिकेट लेयर्स पर Ag-MMT में गोलाकार Ag नैनोकणों के गठन का खुलासा किया, हालांकि कोई बाहरी अपचायक कारक एजेंट कार्यरत नहीं था। हालांकि, Cu और Zn MMT के आदान-प्रदान के मामले में नैनोकणों (NPs) को नहीं देखा गया। संशोधित मिट्टी के खनिजों की जीवाणुनाशक प्रभावकारिता का मूल्यांकन जीवाणु Gram (-) *E. coli*, Gram (+) *S. aureus* और ब्लैक मोल्ड कवक (fungi) *A. niger* के खिलाफ किया गया था जो तीनों प्रकार के एक्सचेंज किए गए एमएमटी के लिए बड़े अवरोधक क्षेत्र दिखाते थे। मिट्टी के खनिजों की न्यूनतम निरोधात्मक एकाग्रता (MIC) का मूल्यांकन किया गया।

MMT जैसे समर्थन पर जमा नैनोकणों (NPs) में स्थिरता, प्रतिक्रियाशीलता और रिलीज विशेषताओं के संदर्भ में असमर्थित नैनोकणों पर फायदे हैं। दूसरा अध्याय MMT (NP-MMT) पर जमा Ag, Cu और ZnO नैनोकणों के संश्लेषण और गुणों पर अध्ययन से संबंधित है। नैनोकण अलंकृत MMT को MMT(M-MMT) के आदान-प्रदान से संश्लेषित किया गया था। विभिन्न रासायनिक और भौतिक अपचायक मार्गों का उपयोग करके एनपी गठन की सुविधा दी गई थी। बीज / नाभिक घनत्व को तय करने और इस तरह अंतिम एनपी आकार का निर्धारण

करने में निर्णायक माध्यम की ताकत को महत्वपूर्ण भूमिका मिली। EDX और ICP-OES विश्लेषण द्वारा धातु तत्वों के पर्याप्त लोडिंग का अवलोकन किया गया, जो M-MMT में ज्ञात सीमाओं की तुलना में बहुत अधिक है। धातु NP-MMT कॉम्प्लेक्स ने *E. coli*, *S. aureus* और *A. niger* के खिलाफ रोगाणुरोधी गतिविधि की स्पष्ट डिग्री का प्रदर्शन किया। NP-MMT में तीन प्रकार के पिंजरों में रोगाणुरोधी गतिविधि की रैंकिंग MMT के आदान-प्रदान के समान है।

MMT प्लेटलेट्स के फैलाव की डिग्री नैनोकम्पोजिट आकृति का फैसला करती है और इसके परिणामी गुणों को नियंत्रित करने में एक महत्वपूर्ण कारक है। तीसरा अध्याय सर्वश्रेष्ठ यांत्रिक और रोगाणुरोधी व्यवहार को प्राप्त करने के लिए डिजाइन (DoE) का उपयोग करके जुड़वां पेंच कंपाउंडिंग की प्रक्रिया मापदंडों के अनुकूलन से संबंधित है। चौथे अध्याय में M-MMT और NP-MMT पर आधारित HDPE नैनोकंपोजिट्स को मेल्ट कंपाउंडिंग द्वारा विकसित किया गया था। नैनोकंपोजिट्स को संश्लेषित MMT सांद्रता (1-5 wt%) को अलग करके तैयार किया गया था और आगे फिलामेंट्स, फिल्मों और मोल्ड किए गए नमूनों को तैयार करने के लिए संसाधित किया गया था। नैनोकम्पोजिट्स ने कम सांद्रता में एक्सफ़ोलीएटेड मिट्टी आकृति विज्ञान दिखाया और TEM और WAXD विश्लेषण द्वारा पुष्टि की गई मिट्टी की उच्च सांद्रता में इंटरकलेटेड और एक्सफ़ोलीएटेड संरचनाओं का मिश्रण। संश्लेषित नैनोकॉम्पोजिट्स पर तर्कसंगत लक्षण वर्णन और क्रिस्टलीकरण अध्ययन भी किए गए थे। यांत्रिक परीक्षण डेटा में तनाव और ब्रेक-वैल्यू में मामूली कमी के साथ MMT को शामिल करने के बाद तन्य शक्ति और मापांक सुधार का पता चला।

पांचवें अध्याय में, HDPE/M-MMT और HDPE/NP-MMT नैनोकम्पोजिट्स के रोगाणुरोधी और साइटोकम्पैटिबल व्यवहार प्रस्तुत किए गए हैं। ढाला नमूना, फिल्म और फिलामेंट की रोगाणुरोधी गतिविधियों के बीच एक तुलनात्मक विश्लेषण उनके रोगाणुरोधी व्यवहार के लिए विभिन्न नमूना रूपों के सहसंबंध का अध्ययन करने के लिए प्रस्तुत किया गया है। HDPE/MMT नैनोकम्पोजिट्स के फिलामेंट्स और फिल्मों ने बड़े सतह क्षेत्र के संपर्क के कारण उच्च प्रसार दरों के कारण संबंधित ढलानों की तुलना में उच्च गतिविधि दिखाई। M-MMT नैनोकॉम्पोजिट्स और NP-MMT नैनोकॉम्पोजिट्स के बीच अंतर भी देखा गया। इसकी लोडिंग के बजाय मुफ्त उपलब्ध आयनों की मात्रा रोगाणुरोधी गतिविधि की सीमा को प्रभावित करने के लिए देखी गई थी। जाहिर है, तीन अलग-अलग प्रकार के धातु आयनों / NP के लिए रोगाणुरोधी व्यवहार में भी महत्वपूर्ण अंतर थे। एंटीमाइक्रोबियल

गतिविधि के तंत्र का अध्ययन टीईएम माइक्रोग्राफ से प्राप्त बैक्टीरिया /NP इंटरैक्शन से किया गया था। M-MMT और NP-MMT से मुक्त धातु आयनों के रिलीज व्यवहार का ICP-MS द्वारा अध्ययन किया गया था। जैसा कि अपेक्षित था, NP-MMT की तुलना में M-MMT में फट रिलीज अधिक प्रमुख है। नैनोकॉम्पोजिट्स की रोगाणुरोधी गतिविधि की स्थायित्व का मूल्यांकन त्वरित स्थितियों में नैनोकॉम्पोजिट्स को उत्तेजित करके समय-मार परख का उपयोग करके किया गया था।

नैनो-टॉक्सिकोलॉजी के लिए बढ़ती चिंताओं और मानव स्वास्थ्य पर इसके प्रभाव को संबोधित करने के लिए, मानव त्वचा और रक्त के साथ अंतरंग संपर्क पर विषाक्तता की मात्रा को निर्धारित करने के लिए HDPE नैनोकॉम्पोजिट्स का मानव कोशिका लाइनों के खिलाफ मूल्यांकन किया गया था। RBC हेमोलिसिस सुरक्षा परख मानव RBC कोशिकाओं के साथ किया गया था और MTT परख में मानव त्वचीय फाइब्रोब्लास्ट कोशिकाओं के साथ साइटोकंपैटिबिलिटी का मूल्यांकन किया गया था। संशोधित मिट्टी ने कुछ हद तक असंगति दिखाई, विशेष रूप से Ag-MMT, हालांकि, निरंतर घर्षण के 48 घंटे के बाद भी उच्चतम लोडेड नैनोकॉम्पोजिट में पूर्ण अनुकूलता प्राप्त हुई। इन-विवो साइटोकंपैटिबिलिटी में स्त्रीग-डावले चूहों पर HDPE नैनोकॉम्पोजिट फिल्मों के साथ शल्य चिकित्सा द्वारा प्रत्यारोपित किया गया। उजागर किए गए भाग से पुनर्प्राप्त त्वचा ऊतक वर्गों का हिस्टोपैथोलॉजिकल सूक्ष्म विश्लेषण 21 दिनों के बाद किया गया था। एपिडर्मिस में कोई असामान्यताएं नहीं देखी गईं, जो HDPE नैनोकॉम्पोजिट्स के संपर्क में आने वाली त्वचा के ऊतकों की पूरी सुरक्षा का संकेत देती हैं।

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