TEMPO INITIATED THIOL-ENE REACTION AS AN EFFICIENT CONJUGATION METHOD FOR THE PREPARATION OF FUNCTIONALIZED POLYMERS AND SURFACES

SUMBUL HAFEEZ

DEPARTMENT OF MATERIALS SCIENCE AND ENGINEERING

INDIAN INSTITUTE OF TECHNOLOGY DELHI

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by

SUMBUL HAFEEZ

Department of Materials Science and Engineering

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CERTIFICATE

This is to certify that the thesis entitled ***"TEMPO Initiated Thiol-Ene Reaction as an Efficient Conjugation Method for the Preparation of Functionalized Polymers and Surfaces"*** *being submitted by* ***Ms. Sumbul Hafeez*** *to the Indian Institute of Technology Delhi for the award of the degree of* ***Doctor of Philosophy*** *is a record of bonafide research work carried out by her. Ms. Sumbul Hafeez has worked under my guidance and supervision and has fulfilled the requirements for the submission of her thesis, which to our knowledge has reached the requisite standard.*

The results contained in this thesis are original and have not been submitted, in part or full, to any University or Institute for the award of any degree or diploma.

Dr. Leena Nebhani

Assistant Professor

Department of Materials Science and Engineering

Indian Institute of Technology Delhi

Hauz Khas, New Delhi, India-110016.

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(SUMBUL HAFEEZ)

ABSTRACT

Since the discovery of the copper catalyzed cycloaddition reaction between azide and alkyne (CuAAC), the scientific community has been motivated to explore additional highly orthogonal and efficient reactions, which can be conducted under mild reaction conditions. Some of the chemistries, which are widely explored are thiol-ene/yne reactions, nucleophilic ring opening reactions, as well as Diels-Alder/hetero Diels-Alder ligations. Thiol-ene reactions can proceed by either a radical mechanism or in a base catalysed fashion (Michael addition). The well-known radical-mediated thiol-ene reaction can be initiated by UV-light in the presence of a photo initiator or by heat in the presence of a thermal initiator. Recently, new pathways for initiating/catalyzing the thiol-ene reaction have been explored such as ultrasound, transition metal polypyridyl photocatalyst, 1,8-diaza bicyclo(5.4.0) undec-7-ene (DBU), base generated *via* visible light, and photo-labile base/super base.

In this thesis, we have studied in detail, a new method for initiating the thiol-ene reaction *via* the use of (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) under ambient conditions, which can be applied for the synthesis of functionalized polymeric microspheres, amphiphilic block copolymers, bioconjugate hybrids as well as for modification of solid substrates. TEMPO is a stable nitroxyl radical, whose stability is due to the steric hindrance of four methyl groups surrounding the nitroxyl functionality. TEMPO is commonly used in nitroxide mediated polymerization (NMP) at high temperatures. The governing mechanism of NMP is the reversible deactivation of the propagating polymer radical by combination with a nitroxide radical. Importantly, using TEMPO as an initiator for the thiol-ene reaction is beneficial as the reaction can be performed under ambient conditions without applying heat or UV light, and therefore opening an avenue for the conjugation of biological entities such as amino acids or peptides, which are heat-sensible.

The optimization of thiol-ene reaction initiated *via* TEMPO was performed by using a variety of thiols, enes, solvents and TEMPO concentration. In order to explore the application of TEMPO initiated thiol-ene reaction for the construction of amphiphilic block copolymers, bioconjugate hybrids, polymeric microspheres and functionalization of solid substrates, firstly well-defined and responsive thiol and ene functionalized polymers were synthesized using reversible addition-fragmentation chain transfer (RAFT) and high-temperature polymerization techniques. The thiol-terminated polymer (poly(*N*-isopropylacrylamide), poly(acrylic acid), polystyrene were prepared by RAFT polymerization of *N*-isopropylacrylamide, acrylic acid and styrene in the presence of RAFT agent and its subsequent aminolysis. The high-temperature polymerization of isobornylacrylate (iBoA) resulted in poly(isobornylacrylate) functionalized with ene moiety (PiBoA-Ene).

In order to synthesize bioconjugates hybrid materials, on the conjugation of poly(*N*-isopropylacrylamide) (PNIPAM)/poly(acrylic acid) (PAA) with cysteine (Cys) (PNIPAM-Cys/PAA-Cys) was performed *via* TEMPO initiated thiol-ene reaction. In order to use cysteine for the preparation of these hybrids materials, firstly functionalization of cysteine with 1,4-bis(acryloyloxy)butane was carried out using TEMPO initiated thiol-ene reaction in an aqueous medium under ambient reaction conditions. The thiol terminated polymers were reacted with Cys-Ene monomer again using TEMPO initiated thiol-ene reaction in an aqueous medium under ambient reaction conditions to yield responsive hybrid bioconjugates. In addition, the antibacterial activity of hybrid materials based on cysteine was evaluated.

The well-defined and responsive amphiphilic block copolymers based on poly(*N*-isopropyl acrylamide)-*b*-poly(isobornyl acrylate) (PNIPAM-*b*-PiBoA), poly(styrene)-*b*-poly(acrylic acid) (PS-*b*-PAA) and poly(isobornyl acrylate)-*b*-poly(acrylic acid) (PiBoA-*b*-PAA) were also synthesized using TEMPO initiated thiol-ene reaction under ambient conditions.

Additionally, modification of silicon wafer was performed by grafting responsive polymer brushes using TEMPO initiated thiol-ene chemistry under mild reaction conditions. The antifouling property of silicon wafer conjugated with cysteine was studied using fluorescence microscopy.

Finally, a series of cross-linked microspheres were synthesized by TEMPO-initiated thiol-ene dispersion polymerization using combinations of a variety of thiols and enes. The surfactant polyvinylpyrrolidone (PVP) loading was varied in order to establish particle dispersion and morphology of cross-linked microspheres. We have synthesized microspheres possessing excess thiol functionalities as well as possessing excess ene functionality, as this will open the possibility to perform a second thiol-ene reaction using TEMPO as an initiator. Therefore, the ability of the microspheres for a second TEMPO-initiated thiol-ene reaction was demonstrated by the ligation of fluorescein-5-maleimide (an ene) to the microspheres' surface containing an excess of thiol functionality and by ligation of cysteine (containing a thiol group) to the microspheres' surface containing an excess of ene functionality.

सार

अज़ाइड और एल्केनी (कॉपरएएसी) के बीच तांबे उत्प्रेरित साइक्लोडोडिशन प्रतिक्रिया की खोज के बाद से, वैज्ञानिक समुदाय को अतिरिक्त उच्च ऑर्थोगोनल और कुशल प्रतिक्रियाओं का पता लगाने के लिए प्रेरित किया गया है, जो हल्के प्रतिक्रिया स्थितियों के तहत आयोजित किया जा सकता है। कुछ केमिस्ट्री, जो कि व्यापक रूप से खोजी गई हैं, वे थिओले-एन / येन प्रतिक्रियाएं, न्यूक्लियोफिलिक रिंग खोलने की प्रतिक्रियाएं हैं, साथ ही डायल्स-एल्डर / हेटेरो डायल्स-एल्डर लिगेशन भी हैं। थिओल-एन प्रतिक्रियाएं या तो एक कट्टरपंथी तंत्र या एक आधार उत्प्रेरित फैशन (माइकल जोड़) में आगे बढ़ सकती हैं। जानी-मानी कट्टरपंथी-मध्यस्थता वाली थिओल-एन प्रतिक्रिया को यूवी-प्रकाश द्वारा फोटो सर्जक की उपस्थिति में या थर्मल थर्मल सर्जक की उपस्थिति में गर्मी से शुरू किया जा सकता है। हाल ही में, थ्योल-ईन प्रतिक्रिया को शुरू करने / उत्प्रेरित करने के नए मार्गों का पता लगाया गया है जैसे कि अल्ट्रासाउंड, संक्रमण धातु पॉलीपीरिडिल फोटोकैटलिस्ट, 1,8- डाईज़ाबाई साईकलो (5.4.0) अन्डेक़-7- ईन (डीबीयू), दृश्य प्रकाश के माध्यम से उत्पन्न आधार , और फोटो-लैब आधार / सुपर बेस।

इस थीसिस में, हमने विस्तार से अध्ययन किया है, (2,2,6,6-टेट्रामिथाइलपिपरीडीन-1-आइल) ऑक्सील (टेम्पो) के उपयोग के माध्यम से थिओल-ईन प्रतिक्रिया को शुरू करने की एक नई विधि, परिवेश स्थितियों के तहत, जो हो सकती है कार्यात्मक पॉलिमेरिक माइक्रोस्फीयर, अम्पिफिलिक ब्लाक को-पॉलीमर, बायोसंकर के साथ ही ठोस सब्सट्रेट के संशोधन के संश्लेषण के लिए आवेदन किया। टेम्पो एक स्थिर नाइट्रॉक्सिल कट्टरपंथी है, जिसकी स्थिरता नाइट्रॉक्सिल कार्यक्षमता के आसपास के चार मिथाइल समूहों के स्टिक बाधा के कारण है। टेम्पो का उपयोग आमतौर पर उच्च तापमान पर नाइट्रॉक्साइड मध्यस्थता बहुलकीकरण (एनएमपी) में किया जाता है। एनएमपी का शासी तंत्र एक नाइट्रॉक्साइड कट्टरपंथी (Radical) के साथ संयोजन द्वारा बहुलक कट्टरपंथी (Rdical) के प्रतिवर्ती निष्क्रिय है। महत्वपूर्ण रूप से, टेम्पो का उपयोग थिओले-एनी प्रतिक्रिया के लिए एक सर्जक के रूप में करना फायदेमंद है क्योंकि प्रतिक्रिया गर्मी या यूवी प्रकाश को लागू किए बिना परिवेश की स्थितियों के तहत की जा सकती है, और इसलिए जैविक संस्थाओं जैसे अमीनो एसिड या पेप्टाइड्स के संयुग्मन के लिए एक एवेन्यू खोलना है, जो गर्मी संवेदी हैं।

टेम्पो के माध्यम से शुरू की गई थ्योल-ईन प्रतिक्रिया का अनुकूलन विभिन्न प्रकार के थ्योल-ईन सॉल्वैंट्स और टेम्पो एकाग्रता का उपयोग करके किया गया था। टेम्पो के आवेदन का पता लगाने के लिए एम्फ़िफ़िलिक ब्लॉक कॉपोलिमर, बायोकॉन्जुगेट हाइड्रिड्स, पॉलीमिक माइक्रोसेफर्स के निर्माण और ठोस सब्सट्रेट के कार्यात्मककरण के लिए पहले से ही अच्छी तरह से परिभाषित और उत्तरदायी थ्योल और एनी फ़ंक्शनलिज्ड पॉलिमर का उपयोग किया गया है, जो प्रतिवर्ती अतिरिक्त-विखंडन श्रृंखला का उपयोग करके संश्लेषित किए गए हैं। स्थानांतरण (आरएएफटी) और उच्च-तापमान बहुलकीकरण तकनीक। थायलेट-टर्मिनेटेड पॉलीमर (पॉली (एन-आइसो प्रोपाइल एकरालामाइड), पाली (एक्रेलिक एसिड), पालीस्टाइरीन राफ्ट एजेंट एन-आइसो प्रोपाइल एकरालामाइड, एक्रिलिक एसिड और स्टाइलिन राफ्ट एजेंट और इसके बाद के एमिनोलिसिस की उपस्थिति में तैयार किए गए थे। एन-आइसो प्रोपाइलबोर्निलएकरिलेट के परिणामस्वरूप पाली प्रोपाइलबोर्निलएकरिलेट का विकास ईन मोयटी के साथ हुआ। बायोकेनजेट्स हाइब्रिड सामग्रियों को संश्लेषित करने के लिए, सिस्टीन (सिस) (पीएनआईपीएऍम-सिस / पीएए-सिस) के साथ पॉली (एन-आइसो प्रोपाइल एकरालामाइड) / पाली (एक्रिलिक एसिड) के संयुग्मन पर टेम्पो के माध्यम से टेम्पो द्वारा शुरू किया गया था। -सबकी प्रतिक्रिया इन संकर सामग्रियों की तैयारी के लिए सिस्टीन का उपयोग करने के लिए, पहले 1,4-बीआईएस (एक्रिलियोक्लोक्सी) ब्यूटेन के साथ सिस्टीन का क्रियात्मकताकरण किया गया था, जो कि एंबियंट रिएक्शन स्थितियों के तहत एक जलीय माध्यम में टेम्पो द्वारा शुरू की गई थियोमो-एनई प्रतिक्रिया का उपयोग करके किया गया था। थ्योल टर्मिनेटेड पॉलिमर को सिस-ईन मोनोमर के साथ फिर से इस्तेमाल किया गया था, टेम्पो का उपयोग करते हुए संवेदनशील हाइब्रिड बायोकॉजगेट्स उपज के लिए परिवेशी प्रतिक्रिया स्थितियों के तहत एक जलीय माध्यम में थ्योल-ईन प्रतिक्रिया शुरू की।

इसके अलावा, सिस्टीन पर आधारित संकर सामग्री की जीवाणुरोधी गतिविधि का मूल्यांकन किया गया था। पाली एन-आइसो प्रोपाइल एकरालामाइड –बी-पाली (आइसोबोर्निल एकरिलेट), पाली (स्टाइरीन) -बी-पाली (एक्रिलिक एसिड) पर आधारित अच्छी तरह से परिभाषित और उत्तरदायी एम्फ़िफ़िलिक ब्लॉक कॉपोलिमर -पीए और पॉली (इसोबोर्निल एक्रिलाट) -बी-पॉली (ऐक्रेलिक एसिड) (पीआईबीओए-बी-पीएए) को भी टेम्पो का उपयोग करके संश्लेषित किया गया था, जो परिवेश स्थितियों के तहत थिओल-एनी प्रतिक्रिया की शुरुआत करता है। इसके अतिरिक्त, सिलिकॉन वेफर के संशोधन को टेम्पो का उपयोग करके उत्तरदायी बहुलक ब्रश ग्राफ्टिंग द्वारा किया गया था, जो हल्के प्रतिक्रिया की स्थिति में थिओल-एन रसायन की शुरुआत करता है। सिस्टीन के साथ संयुग्मित सिलिकॉन वेफर की एंटीफ्लिंग संपत्ति का अध्ययन प्रतिदीप्ति माइक्रोस्कोपी का उपयोग करके किया गया था।

अंत में, क्रॉस-लिंक्ड माइक्रोसेफर्स की एक श्रृंखला को टेम्पो - आरंभित थ्योल-ईन फैलाव बहुलकीकरण द्वारा विभिन्न प्रकार के थिओल्स और एन्स के संयोजन का उपयोग करके संश्लेषित किया गया था। कण फैलाव और क्रॉस-लिंक किए गए माइक्रोसेफर्स के आकारिकी को स्थापित करने के लिए सर्फैक्टेंट पॉलीविनाइलपीरोलिडोन (पीवीपी) लोडिंग विविध था। हमने अधिक थ्योल कार्यक्षमताओं के साथ-साथ अतिरिक्त ईन कार्यक्षमता रखने वाले माइक्रोस्फीयर को संश्लेषित किया है, क्योंकि यह एक सर्जक के रूप में टेम्पो का उपयोग करके दूसरी थ्योल-ईन प्रतिक्रिया करने की संभावना को खोल देगा। इसलिए, एक दूसरे टेम्पो - शुरू की गई हिंसा-एनी प्रतिक्रिया के लिए माइक्रोस्फीयर की क्षमता प्रतिदीप्ति की अधिकता युक्त माइक्रोस्फीयर की सतह पर फ्लुओरेसीन-5-मालेमाइड (एक एनी) के बंधाव द्वारा और सिस्टीन (युक्त युक्त) का प्रदर्शन किया गया था एक थ्योल समूह) माइक्रोस्फीयर की सतह के लिए ईन कार्यक्षमता की अधिकता है।

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LIST OF ABBREVIATION

AIBN	Azobisisobutyronitrile
UV	Ultra-violet
DMF	Dimethylformamide
RAFT	Reversible addition-fragmentation chain transfer
PNIPAM	Poly(*N*-isopropylacrylamide)
PEG	Polyethylene glycol
CRP	Controlled radical polymerization
ATRP	Atom transfer radical polymerization
NMP	Nitroxide mediated polymerization
PDVB	Poly(divinyl benzene)
CuAAC	Copper-catalyzed azide-alkyne cycloaddition
PDI	Polydispersity index
PNIPAM-SH	Thiol-functionalized poly(*N*-isopropylacrylamide)
E.coli	Escherichia coli
S. cerevisiae	Saccharomyces cerevisiae
POSS	Polyhedral silsesquioxane
Tg	Glass transition temperature
AFCT	Addition−fragmentation chain transfer
CCT	Cobalt-mediated catalytic chain transfer

SFRP	Stable free radical polymerization
CSIRO	Commonwealth Scientific and Industrial Research Organization
CTAs	Chain transfer agents
ACVA	4,4′′-Azobis(4-cyanopentanoic acid)
Pn• and Pm•	Propagating radicals
CPADB	2,(2’-Cyanopropyl)dithiobenzoate
CPDB	(4-Cyanopentanoic acid)-4-dithiobenzoate
CDB	Cumyl dithiobenzoate
HDA	Hetero Diels-Alder
TEMPO	2,2,6,6-(tetramethylpiperidin-1-yl)oxyl
NMR	Nuclear Magnetic Resonance
EPR	Electron Paramagnetic Resonance
UV-Visible	Ultraviolet-Visible
XPS	X-ray photoelectron spectroscopy
DSC	Differential scanning calorimetry
CLSM	Confocal laser scanning microscopy
SEM	Scanning electron microscopy
SEC	Size exclusion chromatography
PAA	Poly(acrylicacid)

PS	Polystyrene
PiBoA	Poly(isobornyl acrylate)
Cys	Cysteine
Si-Wafer	Silicon wafer
AFM	Atomic force microscopy
CAM	Contact angle measurement
CA	Contact angle
PVP	polyvinylpyrrolidone
FTIR	Fourier-transform infrared
THF	Tetrahydrofuran
OPET	S-1-Octadecyl S'-(1-phenylethyl) trithiocarbonate)
OMP	(2-Methyl-2-[[(octadecylthio)thioxomethyl] thio]propanoic acid)
AMP	Antimicrobial peptides
MIC	Minimum inhibitory concentration
DLS	Dynamic light scattering
LCST	Lower critical solution temperature
MPTES	3-Mercaptopropyltriethoxysilane
FTIC-albumin	Albumin–fluorescein isothiocyanate conjugate
PBS	Phosphate-buffered

RMS	Root mean square
BDDA	1,4-Butanediol diacrylate
PETTA	Pentaerythritoltetraacrylate
TMPTA	Trimethylolpropane triacrylate
PETMP	Pentaerythritol tetra(3-mercaptopropionate)
TMPMP	Trimethylolpropane tris(3-mercaptopropionate)
DVS	Divinylsulfone