

**STUDIES ON POLY(LACTIC ACID) BASED  
BIODEGRADABLE MICROPARTICLES FOR PROLONGING  
SHELF-LIFE OF FOOD**

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BIODEGRADABLE MICROPARTICLES FOR PROLONGING  
SHELF-LIFE OF FOOD**

*by*

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Submitted

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

to the



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*Dedicated to my family*

## **CERTIFICATE**

This is to certify that the thesis entitled, “**Studies on poly(lactic acid) based biodegradable microparticles for prolonging shelf-life of food**” being submitted by **Mr. Agni Kumar Biswal** to Indian Institute of Technology Delhi for the award of degree of **Doctor of Philosophy** is a record of bonafide research work carried out by him. Mr. Agni Kumar Biswal has worked under my guidance and supervision and has fulfilled the requirements for the submission of this thesis, which to my 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 other University or Institute for the award of any other degree or diploma.

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## ABSTRACT

Controlled-release packaging (CRP) is a rising technology by which actives such as antioxidants and/or antimicrobials can be released in controlled and desirable fashion from the packaging material over a long period of time to prolong the shelf-life of packaged food. Most of the CRP systems developed so far are based on either packaging film or packaged microparticles comprised of actives entrapped in polymeric materials. We were particularly interested in developing particle based systems because of their ease of handling and operations without losing the activity of packaging actives encapsulated in them. To the best of my knowledge, all such microparticles are mostly composed of single polymer and hence, can be useful to deliver single active. In order to release multiple actives (e.g. antibacterial and antioxidant) at desirable rates without having active-active interactions, multilayered particles entrapping different actives in various layers can be quite useful.

In order to achieve the goal, biodegradable multi-layered Poly(lactic-co-glycolic acid)(PLGA)/Poly(l-lactic acid)(PLLA) based dual actives loaded polymeric particles were fabricated and their applications as dual actives (antioxidant and antibacterial) delivery vehicles were explored in Chapter-2. Briefly, 2 kinds of microparticles (bi- and trilayered) made up of PLLA and PLGA with varying viscosity (intrinsic viscosity (I.V.)) were developed using one step emulsion solvent evaporation method. Release study demonstrated that release rate of dual actives (antimicrobial: benzoic acid and antioxidant: tocopherol) was significantly accelerated from tri-layered particles in comparison to bi-layered one. Both sets of particles exhibited long-term antibacterial activity against both *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) bacteria (residual bacterial concentration: 0-2 log(Colony forming unit(CFU)/mL)) as well as antioxidant activity (radical scavenging efficiency: 80-90%) over a period of 60 days. The results show for the first time the feasibility of using multilayered microparticles to prolong the food shelf-life by simultaneous release of dual actives.

To achieve excellent bacterial killing efficiency, instead of using sole benzoic acid (as used in Chapter-2), a minute quantity of mustard oil as second antibacterial was incorporated into PLGA based single phase particles. Surprisingly, PLGA based porous particles were resulted in and these were found to exhibit unusually high antibacterial activity for prolonged period of time. Hence, in Chapter-3, we have focussed on the use of mustard oil in monophasic as well as multi-phasic (multilayered) microparticles to come up with a system having strong antibacterial (not achieved in Chapter-2) as well as antioxidant efficiency. This chapter has been divided into two parts. Part A describes the fabrication of porous PLGA microparticles using water/oil/water (W/O/W) double emulsion technique in presences of a minute quantity of mustard oil. The release of benzoic acid from the porous particles were found to be well-controlled in nature and influenced by surface porosity of the particles that can be manipulated by varying the amount of mustard oil. Strikingly, in liquid medium, porous particles were found completely suppressing the growth of bacteria (100% inhibition) for a prolonged period of 60 days. In a food model system, the shelf life of the watermelon juice was also found to be enhanced by suppressing the growth of the natural microbes in comparison to control. The addition of mustard oil was then extended to multi-layered system. Hence, part B of Chapter-3 focuses on the fabrication of hierarchically porous biodegradable microparticles of various architectures (Janus and core-shell configuration) by adding mustard oil into PLGA/PLLA based multilayered system and evaluation of their applications in prolonging food shelf-life. Synthesis, characterization, and mechanism of formation of various kinds of architecture obtained by varying the mustard oil content in multilayered particles, have been explored in detail. Apart from these, performance of these dual actives (antibacterial and antioxidant) loaded Janus and multi-layered particles in active packaging area were also evaluated for prolonged period of time. Bacterial growth reductions of 2-6 log(CFU/mL) and radical

scavenging efficiency of 70-90% were observed to be maintained for 60 days especially for Janus particles.

In order to develop a triggered release system along with CRP, we have focussed in designing and fabrication of dual actives loaded pH responsive system in Chapter-4. Bi-layered particles with desirable layer compositions (core/shell and vice-versa) were achieved by using acetalated-dextran and PLGA at a mass ratio of 2:1 or 1:2. pH responsiveness was validated by monitoring hydrolytic degradation of these bilayered microparticles in two different pHs (7.4 and 5) by Scanning electron microscope (SEM) and NMR study. A faster release of benzoic acid present in outer layer (acetalated-dextran) was observed in pH 5 for the acetalated-dextran/PLGA (shell/core) system compared to the PLGA/acetalated-dextran (shell/core) system. However, both systems release benzoic acid at similar rates at pH 7.4 but lower than that at pH 5. Similar observations were also found for tocopherol release. The tunable pH responsive behaviour of these microparticles can be potentially utilized for controllable and programmable active delivery not only in acidic food protection but also in drug delivery. Finally, Chapter-5 summarises the investigations and states how these CRP systems can be potentially exploited for prolonging the shelf-life of food.

## सार

नियंत्रित-रिलीज़ पैकेजिंग (CRP) एक उभरती हुई तकनीक है जिसके द्वारा पैकेजिंग सामग्री से लंबे समय तक पैकेजिंग सामग्री से एंटीऑक्सिडेंट और / या एंटीमाइक्रोबायल्स जैसे क्रियाकलापों को नियंत्रित और वांछनीय फैशन में लंबे समय तक जारी किया जा सकता है। अब तक विकसित किए गए अधिकांश सीआरपी सिस्टम या तो पैकेजिंग फिल्म पर आधारित होते हैं या पैक किए गए माइक्रोपार्टिकल्स पॉलीमरिक सामग्रियों में उलझी हुई क्रियाओं से युक्त होते हैं। हम विशेष रूप से कण आधारित प्रणालियों को विकसित करने में रुचि रखते थे क्योंकि पैकेजिंग एक्टिविटीज़ की गतिविधि को खोए बिना उनकी हैंडलिंग और संचालन में आसानी होती थी। मेरे ज्ञान का सबसे अच्छा करने के लिए, ऐसे सभी माइक्रोप्रोटेक्ट्स ज्यादातर एकल बहुलक से बने होते हैं और इसलिए, सक्रिय को वितरित करने के लिए उपयोगी हो सकते हैं। सक्रिय-सक्रिय इंटरैक्शन के बिना वांछनीय दरों पर कई एक्टिविस्ट्स (जैसे जीवाणुरोधी और एंटीऑक्सिडेंट) जारी करने के लिए, विभिन्न परतों में विभिन्न एक्टिविज़ को फंसाने वाले बहुस्तरीय कण काफी उपयोगी हो सकते हैं।

लक्ष्य को प्राप्त करने के लिए, बायोडिग्रेडेबल मल्टी-लेयर्ड पॉली (लैक्टिक-को-ग्लाइकॉलिक एसिड) (PLGA) / Poly (l-lactic acid) (PLLA) आधारित दोहरी एक्टिविटीज़ पॉलीमरिक कणों को गढ़ा गया और उनके अनुप्रयोगों को डुअल एक्टिविटी (एंटीऑक्सिडेंट) के रूप में इस्तेमाल किया गया। और जीवाणुरोधी वितरण वाहनों को अध्याय -2 में खोजा गया था। संक्षेप में, अलग-अलग चिपचिपाहट के साथ PLLA और PLGA से बने 2 प्रकार के माइक्रोपार्टिकल्स (द्वि- और त्रिलयेर) एक चरण इमल्शन विलायक वाष्पीकरण विधि का उपयोग करके विकसित किए गए थे। रिलीज़ के अध्ययन से पता चला है कि द्वि-सक्रिय (एंटीमाइक्रोबियल: बेंजोइक एसिड और एंटीऑक्सिडेंट: टोकोफेरॉल) की रिलीज़ दर द्वि-स्तरित कणों की तुलना में त्रि-स्तरित कणों से काफी तेज थी। कणों के दोनों सेट एस्चेरिचिया कोलाई (ई। कोलाई) और स्टैफिलोकोकस ऑरियस (एस। ऑरियस) बैक्टीरिया (अवशिष्ट जीवाणु एकाग्रता: 0-2 लॉग (कॉलोनी बनाने वाली इकाई / सीएफयू) / एमएल) के खिलाफ दीर्घकालिक जीवाणुरोधी गतिविधि का प्रदर्शन करते हैं। एंटीऑक्सिडेंट गतिविधि के रूप में (कट्टरपंथी मैला ढोने की दक्षता: 80-90%) 60 दिनों की अवधि में। परिणाम पहली बार दोहरे एक्टिविटी के एक साथ रिलीज़ होने से फूड शेल्फ-लाइफ को लम्बा करने के लिए मल्टीलेयर्ड माइक्रोपार्टिकल्स के उपयोग की व्यवहार्यता दिखाते हैं।

केवल बेन्जोइक एसिड (जैसा कि अध्याय -2 में प्रयोग किया जाता है) का उपयोग करने के बजाय उत्कृष्ट बैक्टीरिया की हत्या दक्षता प्राप्त करने के लिए, पीएलजीए आधारित एकल चरण कणों में द्वितीय जीवाणुरोधी के रूप में सरसों के तेल की एक मिनट की मात्रा को शामिल किया गया था। आश्चर्यजनक रूप से, पीएलजीए आधारित झरझरा कणों का परिणाम हुआ और ये लंबे समय तक असामान्य रूप से उच्च जीवाणुरोधी गतिविधि का प्रदर्शन करते पाए गए। इसलिए, अध्याय -3 में, हमने मोनोफैसिक के साथ-साथ मल्टी-फासिक (मल्टीलेयर्ड) माइक्रोपार्टिकल्स में सरसों के

तेल के इस्तेमाल पर ध्यान केंद्रित किया है, जिसमें मजबूत एंटीबैक्टीरियल (अध्याय -2 में प्राप्त नहीं) के साथ-साथ एंटीऑक्सीडेंट दक्षता भी है। इस अध्याय को दो भागों में विभाजित किया गया है। भाग ए में सरसों के तेल की एक

मिनट की मात्रा के संरक्षण में पानी / तेल / पानी (W / O / W) दोहरे इमल्शन तकनीक का उपयोग करके झरझरा पीएलजीए माइक्रोपार्टिकल्स के निर्माण का वर्णन है। झरझरा कणों से बेंजोइक एसिड की रिहाई को प्रकृति में अच्छी तरह से नियंत्रित पाया गया और सरसों के तेल की मात्रा को अलग करके कणों की सतह के छिद्रों से प्रभावित किया जा सकता है। आश्चर्यजनक रूप से, तरल माध्यम में, 60 दिनों की लंबी अवधि के लिए झरझरा कणों को बैक्टीरिया (100% निषेध) के विकास को पूरी तरह से दबाया गया था। एक खाद्य मॉडल प्रणाली में, नियंत्रण की तुलना में प्राकृतिक रोगाणुओं के विकास को दबाने से तरबूज के रस का शेल्फ जीवन भी बढ़ाया गया था। इसके बाद सरसों के तेल को बहुस्तरीय प्रणाली में बढ़ाया गया। इसलिए, अध्याय -3 का भाग बी, पीएलजीए / पीएलएएल आधारित बहुस्तरीय प्रणाली में सरसों के तेल को शामिल करके और विभिन्न खाद्य शेल्फ जीवन में उनके अनुप्रयोगों के मूल्यांकन के द्वारा विभिन्न आर्किटेक्चर (जानूस और कोर-शेल कॉन्फिगरेशन) के पदानुक्रमिक रूप से झरझरा बायोडिग्रेडेबल माइक्रोपार्टिकल्स के निर्माण पर केंद्रित है। मल्टीलेयर्ड कणों में सरसों के तेल की सामग्री को अलग-अलग करके प्राप्त किए गए विभिन्न प्रकार के आर्किटेक्चर के संश्लेषण, लक्षण वर्णन और तंत्र का विस्तार से पता लगाया गया है। इनके अलावा, सक्रिय पैकेजिंग क्षेत्र में लोड किए गए जानूस और बहुस्तरीय कणों में इन दोहरी क्रियाओं (जीवाणुरोधी और एंटीऑक्सिडेंट) के प्रदर्शन का भी लंबे समय तक मूल्यांकन किया गया था। 2-6 लॉग (सीएफयू / एमएल) और 70-90% की कट्टरपंथी मैला ढोने की बैक्टीरियल वृद्धि में 60 दिनों के लिए विशेष रूप से जानूस कणों के लिए बनाए रखा गया था।

सीआरपी के साथ-साथ एक ट्रिगर रिलीज सिस्टम विकसित करने के लिए, हमने अध्याय -4 में दोहरी एक्टीवेटेड पीएच उत्तरदायी प्रणाली के डिजाइन और निर्माण में ध्यान केंद्रित किया है। वांछनीय परत रचनाओं (कोर / शेल और इसके विपरीत) के साथ द्वि-स्तरित कणों को 2: 1 या 1: 2 के बड़े अनुपात में एसीटलेटेड-डेक्सट्रान और पीएलजीए का उपयोग करके प्राप्त किया गया था। स्कैनिंग इलेक्ट्रॉन माइक्रोस्कोप (SEM) और NMR अध्ययन द्वारा दो अलग पीएच (7.4 और 5) में इन bilayered microparticles के हाइड्रोलाइटिक गिरावट की निगरानी के द्वारा पीएच जवाबदेही को मान्य किया गया था। पीएलजीए / एसीटलेटेड-डेक्सट्रान (शेल / कोर) प्रणाली की तुलना में एसीटलेटेड-डेक्सट्रान / पीएलजीए (शेल / कोर) प्रणाली के लिए बाहरी परत (एसीटलेटेड-डेक्सट्रान) में मौजूद बेंजोइक एसिड की तेजी से रिहाई देखी गई। हालांकि, दोनों प्रणालियां पीएच 7.4 पर समान दरों पर बेंजोइक एसिड जारी करती हैं, लेकिन पीएच 5 से कम है। टोकोफेरॉल रिलीज के लिए भी इसी तरह के अवलोकन पाए गए। न केवल अम्लीय खाद्य सुरक्षा में, बल्कि दवा वितरण में भी इन माइक्रोपार्टिकल्स के ट्यून करने योग्य पीएच उत्तरदायी व्यवहार को नियंत्रणीय और प्रोग्राम योग्य सक्रिय वितरण के लिए उपयोग किया जा सकता है। अंत में, अध्याय -5 ने जांच को संक्षेप में कहा है कि इन सीआरपी सिस्टम का भोजन के शेल्फ-जीवन को लंबा करने के लिए कैसे संभावित रूप से शोषण किया जा सकता है।

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## **LIST OF ABBREVIATIONS**

Ac-Dextran	Acetalated-Dextran
AIT	Allyl Isothiocyanate
BA	Benzoic Acid
BCA	2,2'-Bicinchoninate
BCG	Bromocresol Green
CLSM	Confocal Laser Scanning Microscope
CRP	Controlled Release Packaging
CFU	Colony Forming Units
DPPH	2, 2-Diphenyl-1-Picrylhydrazyl
DCM	Dichloromethane
DSC	Differential Scanning Calorimetry
DMSO	Dimethylsulfoxide
<i>E. coli</i>	<i>Escherichia Coli</i>
FDA	Food and Drug Administration
GPC	Gel Permeation Chromatography
HPLC	High-Performance Liquid Chromatography
I.V.	Intrinsic Viscosity
LBL	Layer-by-Layer
LDPE	Low Density Polyethylene
LB	Luria Broth
MTT	Thiazolyl Blue Tetrazolium Bromide
MIC	Minimum Inhibitory Concentration
PCL	Polycaprolactone
PE	Polyethylene
PEO	Polyethylene Oxide
PEA	Polyethylene Alcohol
PEG	Polyethylene Glycol
PET	Polyethylene Terephthalate
PLA/PLLA	Poly(Lactic Acid)/Poly(L-Lactide)
PLGA	Poly(Lactic-Co-Glycolic Acid)
PMMA	Polymethyl Methacrylate
PMSQ	Polymethylsilsequioxane

PP	Polypropylene
PVA	Polyvinyl Alcohol
PPTS	Pyridinium <i>P</i> -Toluenesulfonate
SEM	Scanning Electron Microscope
<i>S. aureus</i>	<i>Staphylococcus Aureus</i>
THF	Tetrahydrofuran
TP	Tocopherol
W/O/W	Water/Oil/Water
WA	Without Actives