Development of Self-assembled Mono Layer of Acid Grafted Chitosan on 316L Stainless Steel

MERLIN.P

(20PCH009)

Thesis Submitted to
Avinashilingam Institute for Home Science and
Higher Education for Women,
Coimbatore- 641043.

In Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE IN CHEMISTRY

MAY - 2022

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Signature of the
Supervisor

26/5/2022
Signature of the
Head of the Department


Acknowledgment

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It is with the choice of blessings and the divine grace of **LORD ALMIGHTY** that any human endeavor is achieved.

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MERLIN P


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LIST OF ABBERIVATION

CH	**Chitosan**
MA	**Mandalic Acid**
PCA	**p-Coumaric Acid**
DPA	**Dipicolinic Acid**
MA-g-CH	**Mandalic Acid grafted Chitosan**
PCA-g-CH	**p-Coumaric Acid grafted Chitosan**
DPA-g-CH	**Dipicolinic Acid grafted Chitosan**
FTIR	**Fourier Transfer Infrared Radiation**
TGA	**Thermal Gravimetric Analysis**
DTG	**Derivative Thermo Gravimetry**
DTA	**Differential Thermal Analysis**
SEM	**Scanning Electron Microscope**

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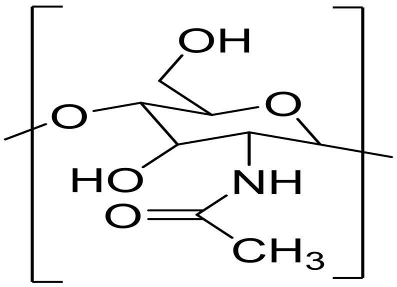
Introduction

1. INTRODUCTION

1.1 Chitin

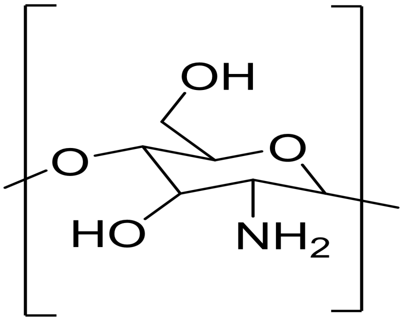
Chitin is the world's second most abundant biopolymer after cellulose **(H.M. Ibrahim *et. al.,* 2015)** . It is found in crustacean such as shells, lobsters, crabs and shrimps **(Grigriy A Mun *et. al.,* 2008).** Chemical structure of chitin is made up of 1-4 linked 2-acetamido-2-deoxy-β-D-glucopyranose. Chitosan differ from chitin by the presence of amino groups , that has been used to synthesize chitosan. The first and foremost step in the extraction of chitosan from chitin is the removal of acetate moiety from chitin. This process is accomplished using hydration that involves amide hydrolysis carried out under alkaline conditions using concentrated sodium hydroxide (NaOH) or through enzymatic hydrolysis in the presence of chitin deacetylase **(Yueyig Wang *et. al.,* 2019)**.



Chitin (1)

1.2 Chitosan

Chitosan (CH), a unique cationic polysaccharide, is a linear macromolecular polymer **(Yueyig Wang *et. al.,* 2019)**. It is a copolymer of N- acetyl D- glucosamine and D-glucosamine.Chitosan is derived from deacetylation of chitin under drastic alkaline conditions or by enzymatic hydrolysis. Chitosan has heterogeneous structure, made up of both 1-4 linked 2-acetamido-2-deoxy-β-D-glucopyranose as well as 2-amino-2-deoxy- β-D-glucopyranose. Chitosan is made up of linear β-(1–4) glycosidic linkages. These linkages in chitosan are similar in structure to cellulose **(Yueyig Wang *et. al.,* 2019)**.



Chitosan (2)

Molecular weight of chitosan is typically between 300 – 1000 kDa depending on the source of chitin. It is only soluble in acidic media. Chitosan is the only positively charged, naturally occurring polysaccharide **(H.M. Ibrahim *et. al.,* 2015)**.

Chitosan is a renewable polymers in a number of forms such as particles, films, gels, membranes or scaffolds for different targeted applications. It is a highly basic polysaccharide as opposed to the other polymers such as agar, pectin, dextrin, agarose, carragenas and cellulose.
Chitosan has linear amino polysaccharide with high nitrogen content, rigid D-glucosamine structure, high crystallinity and hydrophilicity. It's a weak base and deprotonated amino group acts as a powerful nucleophile. It has a capacity to form hydrogen bonds intermolecularly and high viscosity. It forms salts with organic and inorganic acids. Chitosan has chelating and complexity properties, ionic conductivity and numerous reactive groups for chemical activation and cross-linking **(Madihally S V *et. al.,* 1999)**.

The amino groups in chitosan is a base. It is not dissolve in water and basic solution and soluble in dilute acids with the pH of < 6. The practical applications of chitin are extremely limited due to its poor solubility. **(Madihally S V *et. al.,* 1999)**. It has both reactive amino and hydroxyl groups that can be used to chemically alter its properties under mild reaction conditions. It is a bio-adsorber with gel forming ability **(Croisier F *et. al.,* 2013, Rinaudo M 2006)**.

Chitosan with protonated amino groups becomes a polycation that can subsequently form ionic complexes with a wide variety of natural or synthetic anionic species **(Madihally S V *et. al.,* 1999)**, such as lipids, proteins, DNA and some negatively charged synthetic polymers as poly acrylic acid. As a matter of fact, chitosan is the only positively charged, naturally occurring polysaccharide **(Pavinatto F J *et. al.,* 2010)**. Chitosan molecules have both amino and hydroxyl groups so that it can form stable covalent bonds via several reactions such as etherification, esterification and reductive amination reactions **(Croisier F *et. al.,* 2013, Rinaudo M 2006).**

1.3 Graft copolymerization

The presence of active functional primary amino groups in chitosan provides a specific platform for side group attachment under mild reaction conditions depending upon the targeted applications. The presence of these side groups on a chitosan backbone has been found to provide flexibility to the given material with specific functionality. These groups are also responsible for changing the biological and physical properties. Different types of graft copolymerization reactions used to alter the properties of chitosan **(Zhikuan Yang *et. al.,* 1999).**

The C_2 amino group in chitosan was protected from the reaction between benzaldehyde and chitosan to form N-benzylidene chitosan (CBT). Then, the chitosan– benzaldehyde–mesocyclic diamine crown ether (CTBA) was prepared by reacting CTBA and a dilute ethanol hydrochloride solution to give a novel kind of grafted chitosan-crown ether (CTDA)**(Zhikuan Yang *et. al.,* 1999).**

1.4 Applications of chitosan

Chitosan have remarkable antibacterial activity **(Croisier F *et. al.,* 2013, Ong S Y *et. al.,*2008)**, along with antifungal (Aranaz I et. al.,2009), mucoadhesive (Lehr C M et. al.,1992), analgesic **(Aranaz I *et. al.,*2009)** and haemostatic properties **(Yang J *et. al.,*2008)**. It can be biodegraded into non toxic residues **(Baghevi Khoulenjani S *et. al.,*2009,Varum K M *et. al.,*1997)** the rate of its degradation being highly related to the molecular mass of the polymer and its deacetylation degree – and has proved to some extent biocompatibility with physiological medium **(Vande Vord P J *et. al.,*2002,Sashiwa H *et. al.,*2004).** It tends to form complexes with metal ions which can be used for waste water treatment and purification **(Rinaudo M 2006)**.

All these singular features make chitosan an outstanding candidate for biomedical applications. Chitosan shows two ways to inhibit microbial growth. The first way is, positively charge on chitosan binds with negative charge on the bacterial cell surface. It alters the permeability and leaks solutes outside the cells. The second way is, binding with DNA, which hinder RNA synthesis. In the biomedical field, chitosan has been found to increase the attachment of the implant to cells**(H M Ibrahim *et. al.,*2015).**

Chitosan has special features such as biocompatibility, viscosity, biodegradability, polyelectrolyte behaviour, non- toxicity, low immunogenicity, film forming and metal chelating ability **(Yueying Wang *et. al.,*2008, Jun Liu *et. al.,*2017)** .To improve biomedical applications, chitosan is conjugated with compounds such as polyphenols, acids, aldehydes, ketones and amines etc. Compared with native chitosan, polyphenol – chitosan conjugates show various enhanced functional properties such as increased drug delivery, act as anti-diabetic agent and antioxidant additive, used in tissue engineering, food additives and packaging sectors **(Qiaobin Hu Yang Chao Luo 2016).**

The rapid healing of endothelium on the stent surface in a simpler way by coating the stent surface with a cytophilic natural polymer layer. Chitosan and heparin used for their oppositely charged polymer electrolyte characteristic to form a stent coating through a layer-by-layer (LBL) self-assembly method. LBL self-assembly technique is a relatively new and easy approach to the preparation of complex polymer membranes, which takes advantage of the static or hydrogen-bond interactions between different kinds of macromolecules. This kind of membrane, although very thin, retains the original properties of the component polymers **(Sheng Meng *et. al.,*2009).**

The super absorbent resins grafted with chitosan can absorb aqueous solutions up to hundreds of times their own dry weight and should have the antibacterial activities. Therefore, the super absorbent resin can be used in infant diapers, feminine hygiene products, and agriculture. An effective approach to modify swelling behavior of chitosan resin in various pHs is graft polymerization of vinylic monomers such as acrylic acid, acrylamide, acrylonitrile onto chitosan **(Ge Huacai *et. al.*,2006)**.

Chitosan also displays antimicrobial activity against a wide range of microorganisms such as algae, fungi and bacteria . Furthermore, it was shown that treatment with chitosan can efficiently reduce or prevent bacterial biofilm formation, both in vitro and in vivo **(A Silva-Das *et. al.*,2013).**

The introduction of gelatin into a matrix of chitosan is one of the approaches being investigated to enhance further the cell response to chitosan . Nanoparticles, made of gelatin, for example, can be tailored in terms of degradation behavior and they can serve as addition to chitosan matrices. Moreover through hydrophobic and electrostatic interactions, drug delivery properties of gelatin nanoparticles can be controlled and they are suited to be used as drug carriers embedded in a matrix of chitosan **(Svenja Heise *et. al.*,2019).**

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Chitosan is an inexpensive polysaccharide with a long-chain backbone composed of N-acetyl glucosamine. It has been utilized in soil amendment and seed treatment, as well as for foliar treatment to control the fungi,Fusarium oxysporum, Fusarium acuminatum, Cylindrocladium floridanum, and Aspergillus flavus **(Ruixiu Jia *et. al.*,2019).**

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The application of chitosan as a bioactive substance to control postharvest fungal disease has also drawn great attention due to public concerns about food safety, overuse of synthesized chemicals, fungicide residue, and emergence of fungicide-tolerant pathogens. However, the remarkably poor solubility of chitosan, caused by the high crystallinity owing to the existence of hydrogen bonds and acetamido groups, limits its wide application. Therefore, it is necessary to modify the structure of pristine chitosan to improve its solubility and other properties **(Ruixiu Jia *et. al.*,2019).**

Chitosan is considered an excellent biomaterial for edible coatings. Previous studies proved that chitosan-based coatings are able to improve the quality and extend the shelf life of fruit and introducing bioactive compounds into edible coatings can further enhance the preservative effect. Therefore, many studies are focusing on seeking and inserting suitable bioactive compounds onto Chitosan to prepare edible coatings **(Yingjun Jing *et. al.*,2019).**

Procyanidin (PC), a member of natural polyphenols in plants possess the potent antioxidant capacity due to its abundant phenolic hydroxyl groups. Conjugation of poly-phenols onto CS can enhance dramatically the antioxidant activity of CS . Thus, conjugation of PC onto CS may be an available way to prepare a suitable biomaterial for edible coatings **(Yingjun Jing *et. al.*,2019).**

Chitosan has been recently focused attention because it is one of naturally occurring, biodegradable polymers and shows various pharmacological activities such as antifungal, anti-allergic,anti-tumor, immune activating effects and so on. Therefore, it was expected to use chitosan as medical materials as well as chitin. Some chitosan-containing textiles were developed and were already used for commercial cloths expecting its antifungal activity. Chitosan, however, dissolves in various aqueous solutions containing organic and inorganic acids because it is only basic polymer occurring naturally. It is possible to use these chitosan solutions for preparing films or fibers by evaporating the water **(Yasua Sakai *et. al.*,2001).**


OBJECTIVES

OBJECTIVE

The chitosan synthesised from chitin via hydrolysis of acetamide groups reported by Inmaculada Aranaz et al., in 2009. In 2016, Richa Panwar et al., reported synthesis of ferulic acid grafted chitosan by dissolving chitosan in 10% acetic acid using tripolyphasphate as initiator. Methacrylic acid grafted carboxymethyl chitosan was developed by ammonium persulfate as intiator, reported by Tao Sun et al., in 2002. The flim of sodium alginate and carboxymethyl chitosan-ZnO nanoparticles developed by adding glycerol to ultrasonified homogenous acetic acid solution (Wong et al., 2019) with the above mentioned views, the present work aims:

- To study the graft polymer formation of chitosan with various acids such as mandalic acid, p- coumaric acid and dipicolinic acid.
- To prepare sol gel of acid grafted chitosan, using glycerol as binder.
- To form self-assembled monolayer film on 316L stainless steel with different pH and drying method.
- To characterize and predict the structure of compounds by FTIR technique.
- To determine the stability of the compounds by TGA.
- To evaluate the thickness of the films by SEM.

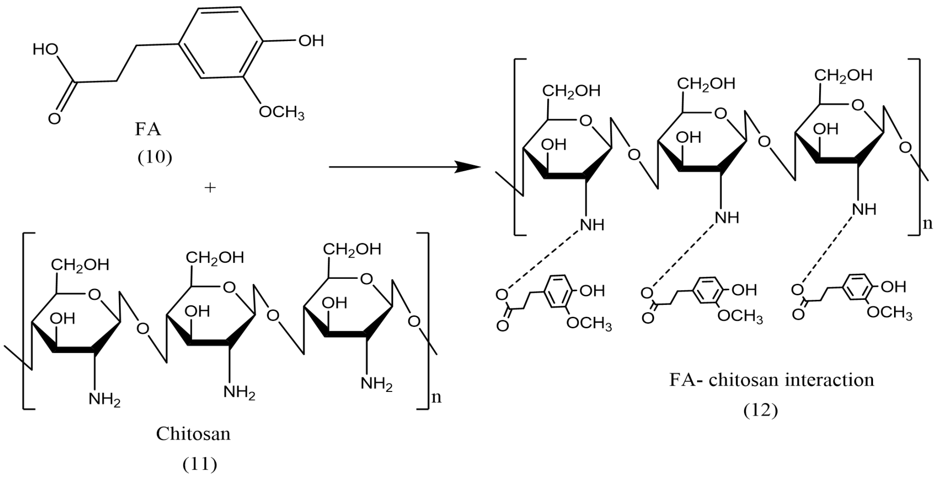

Literature Review

2. REVIEW OF LITERATURE

Fei Chen *et. al* ., (2009) investigated antioxidant and antibacterial activities of Eugenol and carvacrol-grafted chitosan nanoparticles. In this study, they have synthesized eugenol-grafted chitosan nanoparticles and carvacrol-grafted chitosan nanoparticles from eugenol aldehyde and carvacrol aldehyde with methanol and chitosan nanoparticles as Schiff base reaction. The DPPH assay and Minimum Inhibitory Concentration (MIC) test confirmed that the grafted polymer has the ability of antioxidant and antimicrobial activity.

Issam Sebti *et. al.,* (2006) reported Chitosan Polymer as Bioactive coating and film against Aspergillus niger Contamination. Chitosan polymeric In this study, chitosan polymeric films was formed by dispersing chitosan in 0.5% aqueous 1M acetic acid solution. 1M NaOH was used to get desired pH. The precipitation was filtered. The filtrate (chitosan solution) was poured onto a polypropylene plate to get a uniform layered film.Futher, the films dried in oven for 2hrs at 60°C. The chitosan films were studied antimicrobial activity sgainst Aspergillus niger. The report showed chitosan films limits the food degradation and improves the food shelf life.

Richa Panwar *et. al.,* (2016) studied the efficiency of ferulic acid encapsulated chitosan nanoparticles against candida albicans biofilm. In this study they have synthesized ferulic acid chitosan (12) through adding ferulic acid (10) on to chitosan (11) solution using tripolyphosphate (TPP) as initiator.



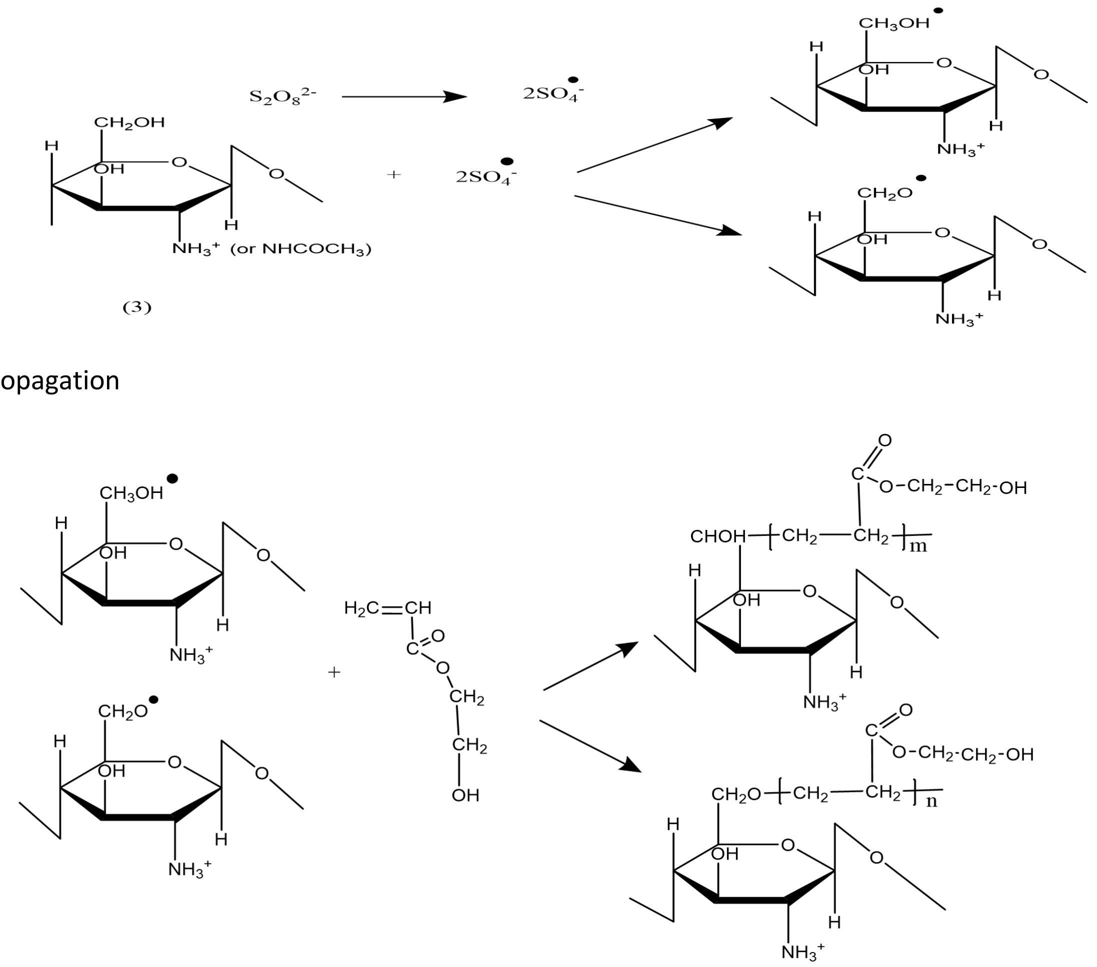
FA
(10)

Chitosan
(11)

FA- chitosan interaction
(12)

Grigoriy A. Mun *et. al* ., (2008) investigated the studies on graft copolymerization of 2-hydroxyethyl acrylate onto chitosan. In this study, they grafted 2- hydroxyethyl acrylate (HEA) on chitosan (4) from the reaction of hydroxyl ethyl acrylate using ammonium persulphate as an initiator in the chitosan (3) solution and precipitated in acetone. Further the grafting reaction was varied by using different initial concentrations of the monomer, initiator and chitosan as well as time and temperature of the reaction. It affects the grafting percentage, grafting efficacy and dynamic viscosity. The HEA grafted chitosan results increased solubility in water.

Initiation

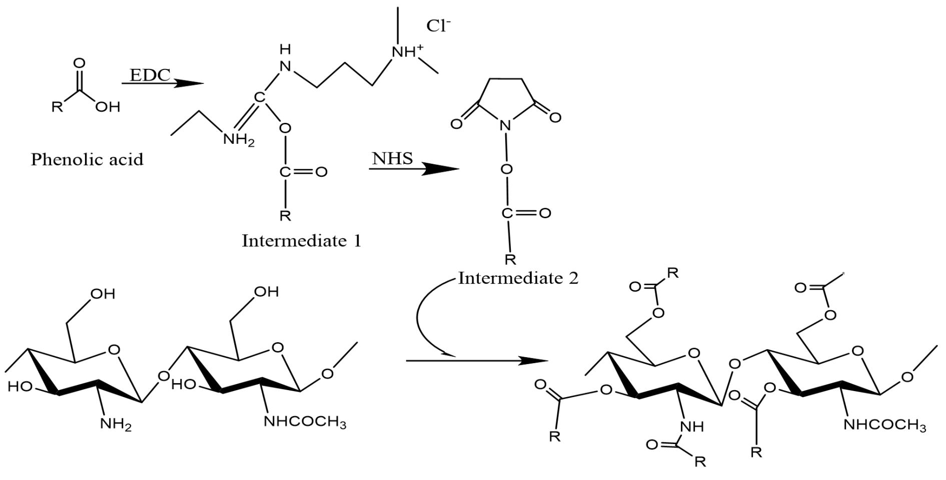


Termination

$(\text{Chitosan-g-PHEA})_n + (\text{Chitosan-g-PHEA})_m \longrightarrow (\text{Chitosan-g-PHA})_{n+m}$

(4)

Jun Liu *et. al .,* (2017) studied the synthesis, characterization, bioactivity and potential application of phenolic acid grafted chitosan – a review. In this study, they reviewed synthesis of various phenolic acid grafted chitosan (6) using 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) and dicyclohexyl carbodimide as coupling reagents in grafting reaction between various phenolic acid such as gallic acid and chitosan (5).



Chitosan

(5)

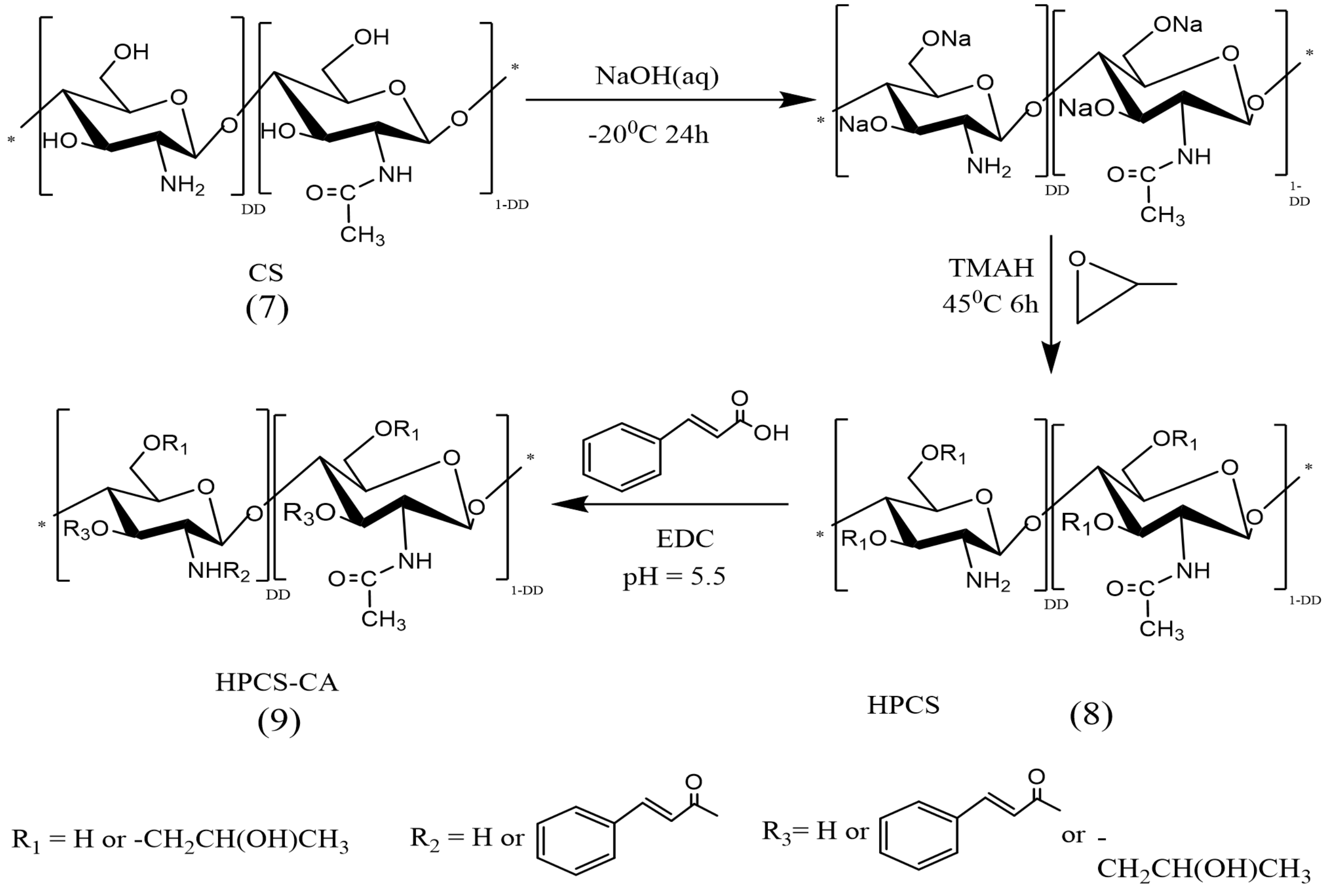
Phenolic acid-g-chitosan

(6)

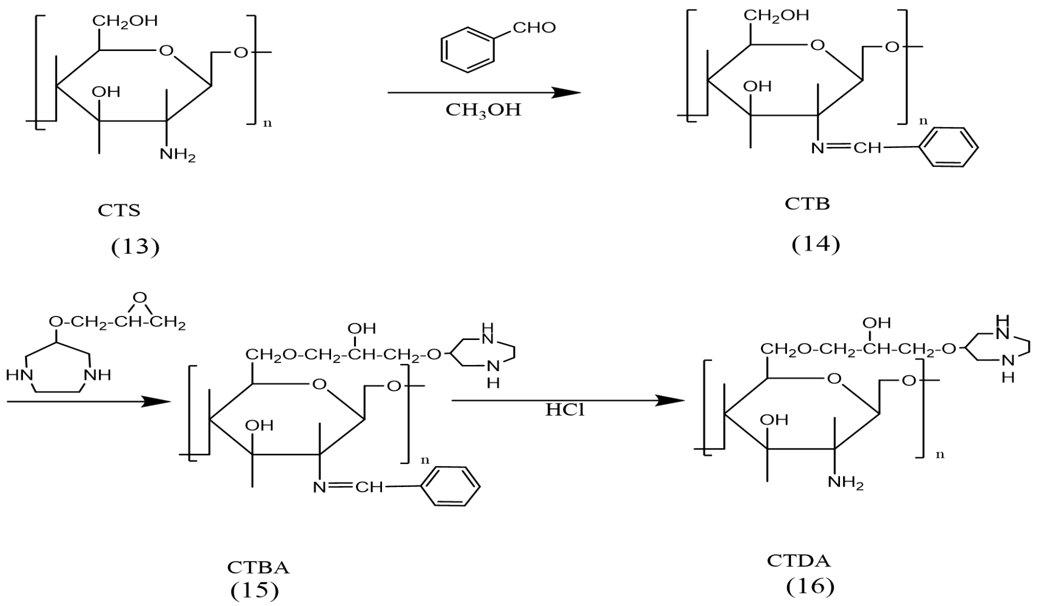
Fatemehsadat Pishbin *et. al.,* (2014) studied the Electrophoretic Deposition of Gentamicin-Loaded Bioactive Glass/ Chitosan Composite Coatings for Orthopaedic Implants. In this study, they have deposited Getamicin, bio-active glass and chitosan on 316L SS using Electrophoretic method. The 316L SS act as cathode and a gold counter electrode is used. The getamicin bio-active glass chitosan solution was used as suspension. When constant voltage is applied, deposition occurs. Gentamicin inhibits the bacterial growth for 2 days under analysis.

Hsu-Wei Fang *et. al.,* (2008) studied the Dip coating assisted polylactic acid deposition on steel surface: Film thickness affected by drag force and gravity. In this study they have designed a prepared stainless steel (316L) coated in a poly(DL-Lactic) acid solution by a controller. This apparatus able to move a clamp at constant speed. The coated 316L stainless steel plates were analyzed under surface profilometer. This results the gravity effect, drag force decides the thickness of the film.

Lin Yue *et. al*., (2021) analyzed the preparation, characterization and antibiofilm activity of cinnamic acid conjugated hydroxypropyl chitosan derivatives. in his study they have developed cinnamic acid conjugated hydroxy propyl chitosan derivatives (HPCS-CA) (9), primary hydroxypropyl chitosan (HPCS (8)) was prepared by chitosan (7) solution (in NaOH) with tetramethyl ammonium hydroxide (TMAH), isopropyl alcohol and propylene oxide, secondly HPCS-CA is synthesized by dissolving cinnamic acid and HPCS (8) in DMSO using EDC as initiator.



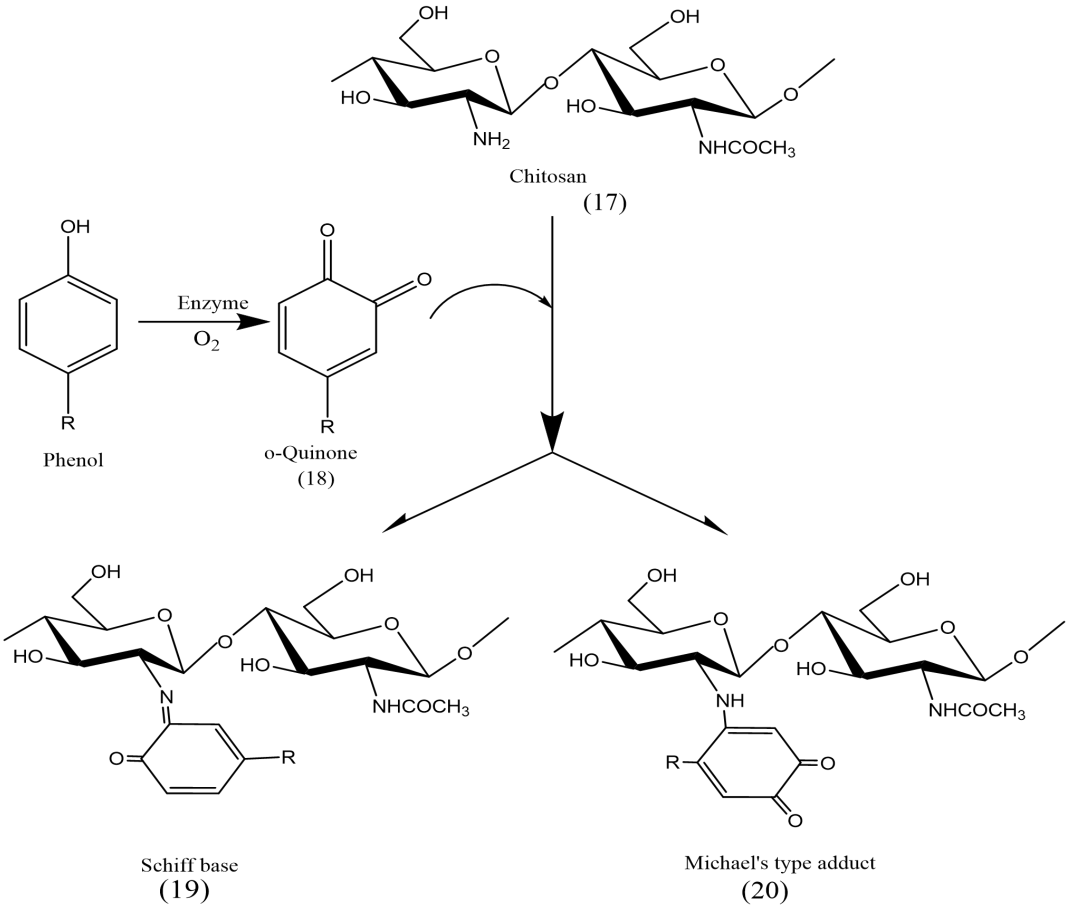
Zhikuan Yang *et. al.,* (1999) studied the synthesis and adsorption properties for metal ions of mesocyclic diamine grafted chitosan-crown. In this study, they have synthesized chitosan-crown ether (CTDA) (16) via Schiff base reaction between N-Benzaldehyde chitosan (CTB) (14) powder (which is the product of chitosan (13), benzaldehyde and methanol) treated with epoxy mesocyclic diamine (EMDA) and dilute ethanoic hydrochloride solution.



Md Nur Alam *et. al.*, (2018) analyzed the natural cellulose- chitosan crosslinked super absorbent hydrogels with superior swelling properties. In this study they have developed cellulose-chitosan crosslinked hydrogels by heating cellulose (chemically modified by periodate oxidation and chlorite oxidation) and carboxy methylated chitosan at 60°C for 1 hour and kept for 4 to 6 hours at room temperature. No catalyst have been used in this synthesis.

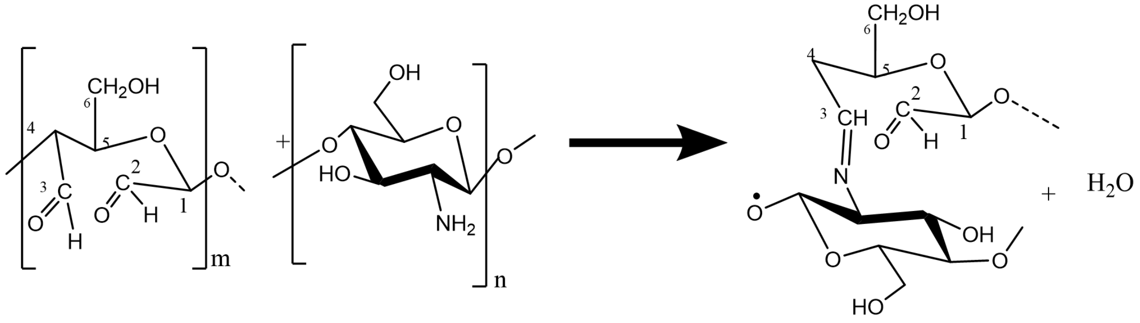
Eunsoo Kim *et. al.,* (2020) studied the chitosan-grafted-poly (aniline-co-anthranilic acid)) as a water-soluble binder to form 3D structures for Si anodes. In this study they have synthesized chitosan-grafted poly (aniline-co-anthranilic acid) from the reaction of chitosan, aniline and anthranilic acid using ammonium persulfate under nitrogen atmosphere, neutralized with sodium hydroxide, purified by acetone and NMP.

Jun Liu ***et. al.,*** **(2017)** studied the synthesis, characterization, bioactivity and potential application of phenolic acid grafted chitosan – a review. In this study, they have reviewed they reviewed synthesis of various phenolic acid grafted chitosan from the reaction of phenolic acid (18) such as gallic acid, caffeic acid, ferulic acid and chitosan (17) using enzymes such as laccase, peroxidase and grosinase as catalyst. The products are mostly sciff base (19) and michaels's adduct (20) of chitosan and phenolic acid.



Ge Huacai ***et. al.,*** **(2006)** studied the graft copolymerization of chitosan with acrylic acid under microwave irradiation and its water absorbency. In this study, they have developed acrylic acid grafted onto chitosan, by dissolving chitosan in acetic acid and reacted with acrylic acid, using ammonium ferous sulfate as initiator, crosslinked using N,N' – methylene bis acrylamide (NBA) solution, neutralized with NaOH solution under microwave.

Tamer M. Tamer *et. al.*, (2015) investigated the development of thermo-sensitive poly N-isopropyl acrylamide grafted chitosan derivatives. In this study they have synthesized poly-n-isopropyl acrylamide (PNIP AM) aminated chitosan (AMCH) (23) by free radical polymerization between n-isopropyl acrylamide (21) and aminated chitosan (22) using potassium persulphate (KPS) as initiator under nitrogen atmosphere.



(21)
Cellulose aldehyde

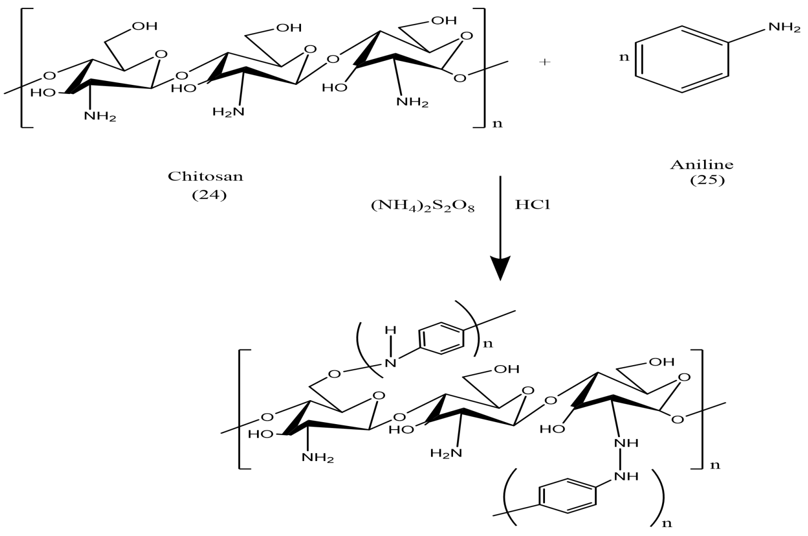
(22)
Chitosan

(23)
Imino bond

Rafael de Oliveria Pedro Carla *C et. Al.,* (2016) studied the Syntheses and characterization of amphiphilic quaternary ammonium chitosan derivatives In this study, they have synthesised amphiphilic derivatives of chitosan by grafting chitosan and triethylammonium bromide (BPA) in aqueous NaOH solution produce quaternized chitosan which is treated with dodecyl aldehyde (DDA) using sodium cyanoborohydride as reagent.

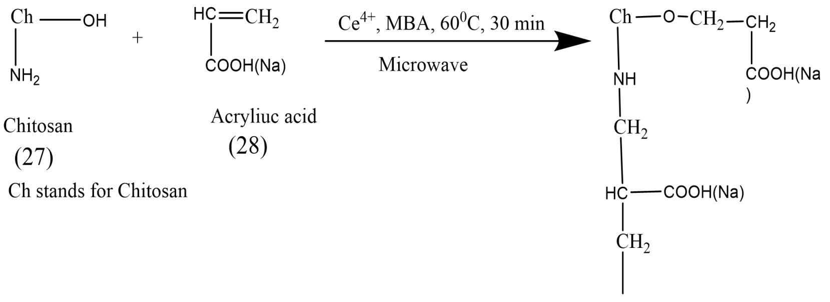
K.V. Vishnu *et. al.,* (2017) studied the Microencapsulation of sardine oil: application of vanillic acid grafted chitosan as a bio-functional wall material In this study, they have developed vanillic acid grafted chitosan by the interaction between chitosan (dissolved in acetic acid) and vanillic acid (dissolved in ethanol) under nitrogen atmosphere.

A. Tiwari *et. al.,* (2007) studied the synthesis and characterization of electrical conducting chitosan-graft-polyaniline (26). In this study, they have synthesized chitosan-graft-polyaniline from the reaction chitosan (24) and aniline (25) (dissolved in aqueous HCl) using ammonium persulfate, neutralized with sodium hydroxide, purified by N-methyl pyrrolidinone.



Chitosan-graft-polyaniline
(26)

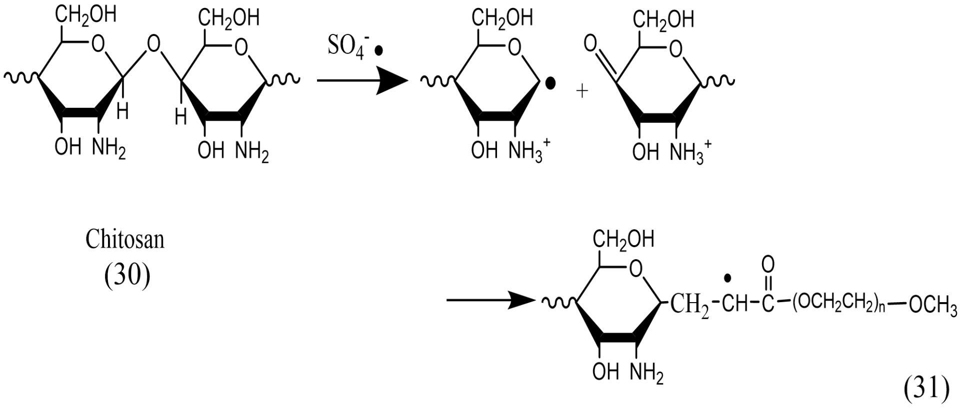
Khaled F. El-Tahlawy *et. al.,* (2006) investigated the preparation and application of chitosan/poly(methacrylic acid) graft copolymer. In this study they have grafted poly(methacrylic acid) graft copolymer (29) by the reaction between chitosan (27) (dissolved in equivalent amount of water and formic acid) and methacrylic acid (28), using 2,2-azobis (2-methyl propionitrile) as polymerization initiator.



Chitosan- graft - acrylic acid
(29)

F. Gan ji ***et. al.,*** **(2008)** studied the synthesis and characterization of a new thermosensitive chitosan- PEG diblock polymer. In this study, they have synthesized chitosan-PEG-diblock copolymer (31) by reacting chitosan (30) (dissolved in acetic acid) poly ethylene glycol macromere (33) (using potassium per sulfate (KPS) in the reaction under nitrogen atmosphere and neutralized sodium hydroxide.

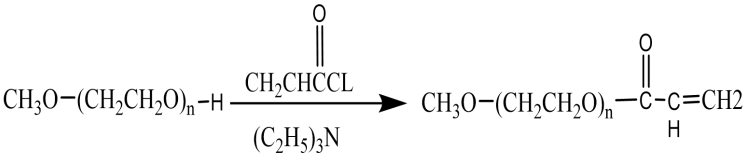
$$K_2S_2O_8 \xrightarrow[60^0C,\ 30min]{N_2} 2\ SO_4^{-}\bullet\ + 2K^+$$



Chitosan
(30)

(31)

Chitosan-PEG block copolymer



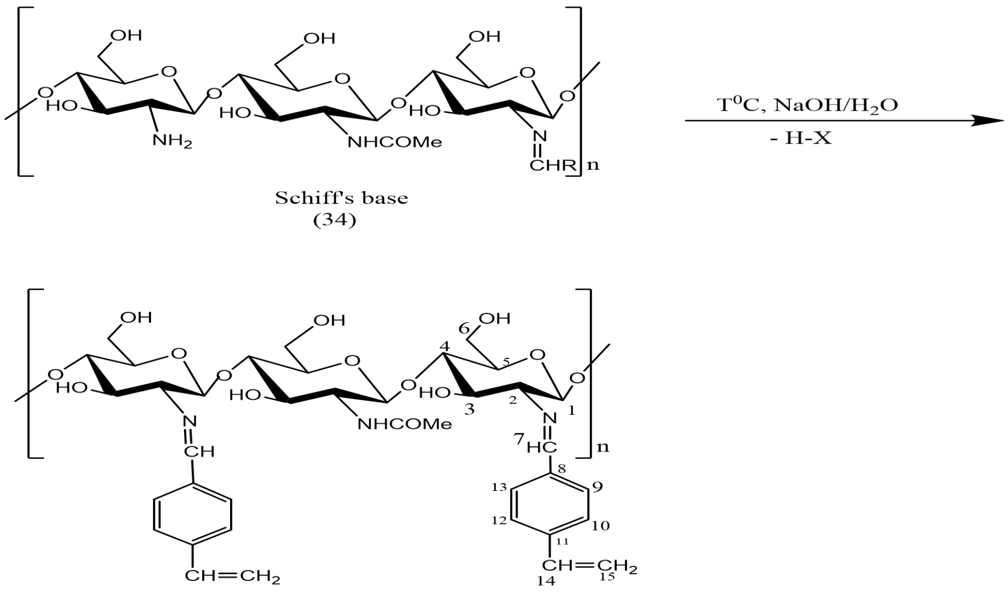
$$CH_3O-(CH_2CH_2O)_n-H \xrightarrow[(C_2H_5)_3N]{CH_2CH\overset{O}{\overset{\|}{C}}CL} CH_3O-(CH_2CH_2O)_n-\overset{O}{\overset{\|}{C}}-\underset{H}{C}=CH2$$

MPEG
(32)

PEG macromer
(33)

Chen Li ***et. al.,*** **(2017)** studied the Preparation of chitosan-ferulic acid conjugate: Structure characterization and in the application of pharmaceuticals In this study, they have synthesised chitosan-ferulic acid conjugate from the reaction of chitosan (dissolved in acetic acid) and ferulic acid in a nitrogen flushed three necked round bottom flask.

Vasak B. Gavalyan *et. al.,* (2016) investigated the Synthesis and characterization of new chitosan-based Schiff base compounds. In this study, they have synthesized chitosan Schiff base (35) by reaction of chitosan (34) with 4-(2-chloroethyl) benzaldehyde and 4-(2- bromoehtyl) benzaldehyde by maintaining the temperature using NaOH, water has been eliminated.



Schiff's base
(34)

(35)

$T^0C = 10.$

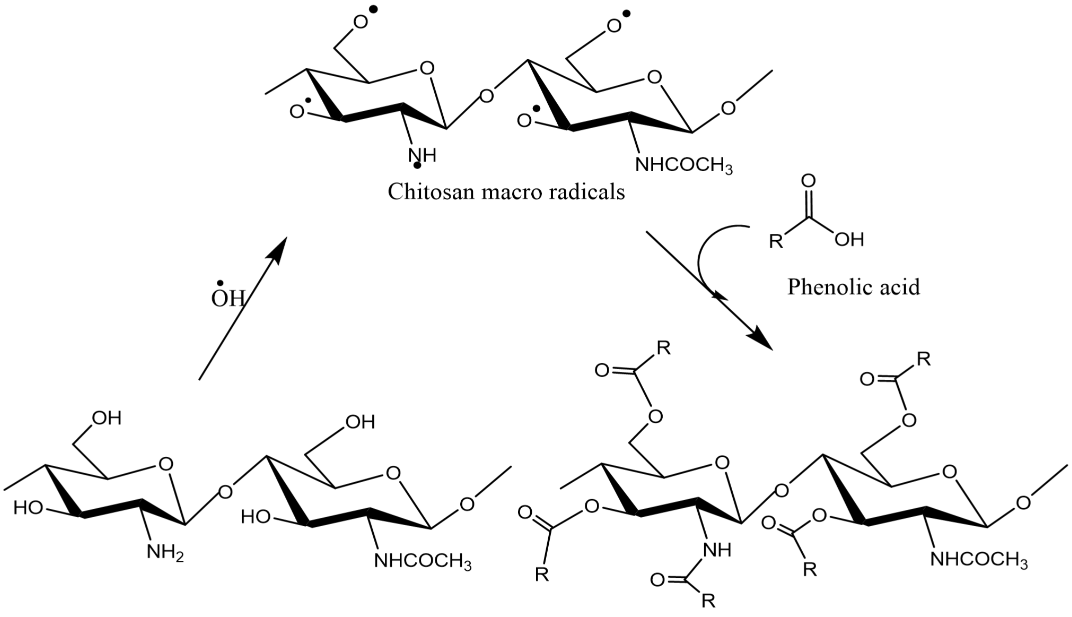
$NaOH/H_2O = 3.75g/19$ ml.

H-X = HBr; HCl.

Wenming Xie *et. al.,(*2001) analysed the antioxidant activity of water-soluble chitosan derivatives. In this study, they have investigated about the antioxidant activity of hydroxypropyl (HPCT) or carboxymethyl (CMCTS) grafted chitosan, which is prepared by graft copolymerization of HPCT or CMCTS and chitosan solution using ammonium persulfate as initiator under nitrogen atmosphere.

Pengiu Lv *et. al.*, (2009) analysed the studies on graft copolymerization of chitosan with acrylonitrile by the redox system. In this study, an acrylonitrile-chitosan conjugate is synthesised by the radical polymerization between chitosan and acrylonitrile solution using a pair of initiator such as ammonium persulphate and sodium thiosulfate.

Jun Liu *et. al.,* (2017) studied the synthesis, characterization, bioactivity and potential application of phenolic acid grafted chitosan – a review. In this study, they have synthesized phenolic acid-g- chitosan (37) via free radical mediated reaction, ceric ammonium nitrate, potassium persulfate and ascorbic acid/hydrogen peroxide redox pair initiate the grafting copolymerization reaction between phenolic acid and chitosan (36).

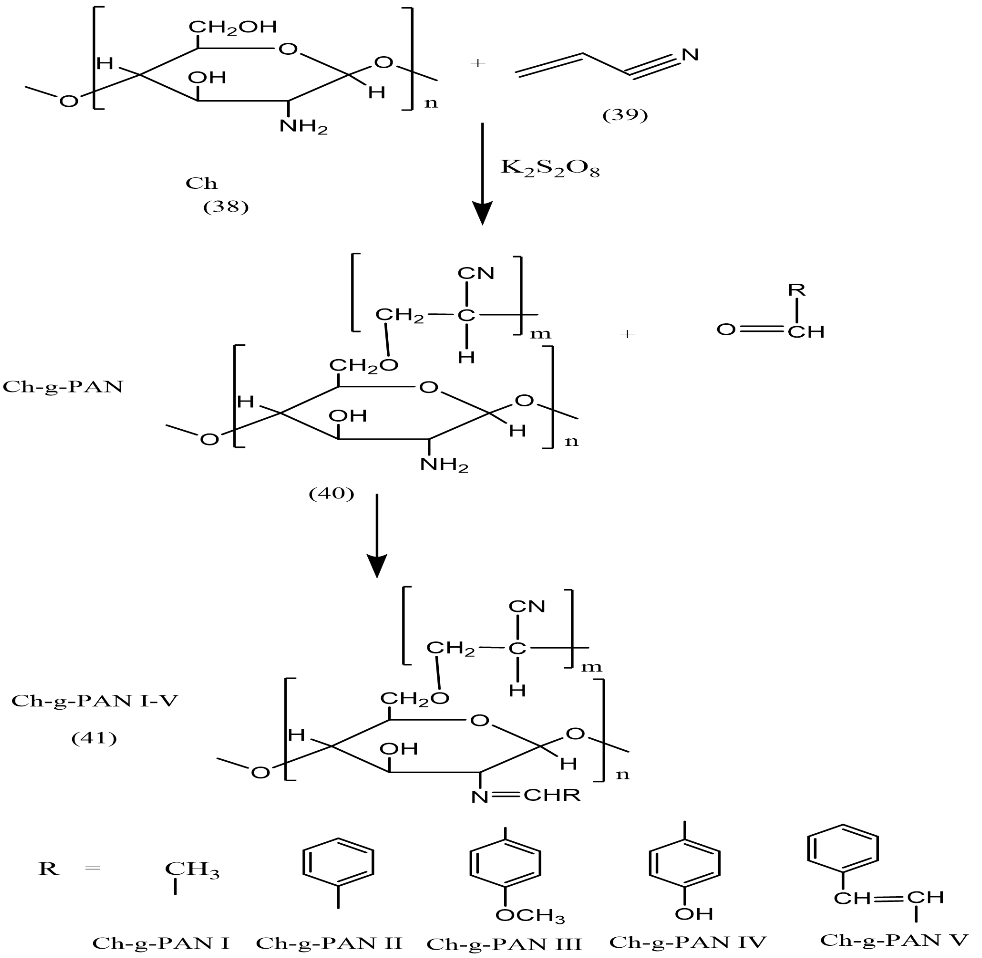


Chitosan
(36)

Phenolic acid-g-chitosan
(37)

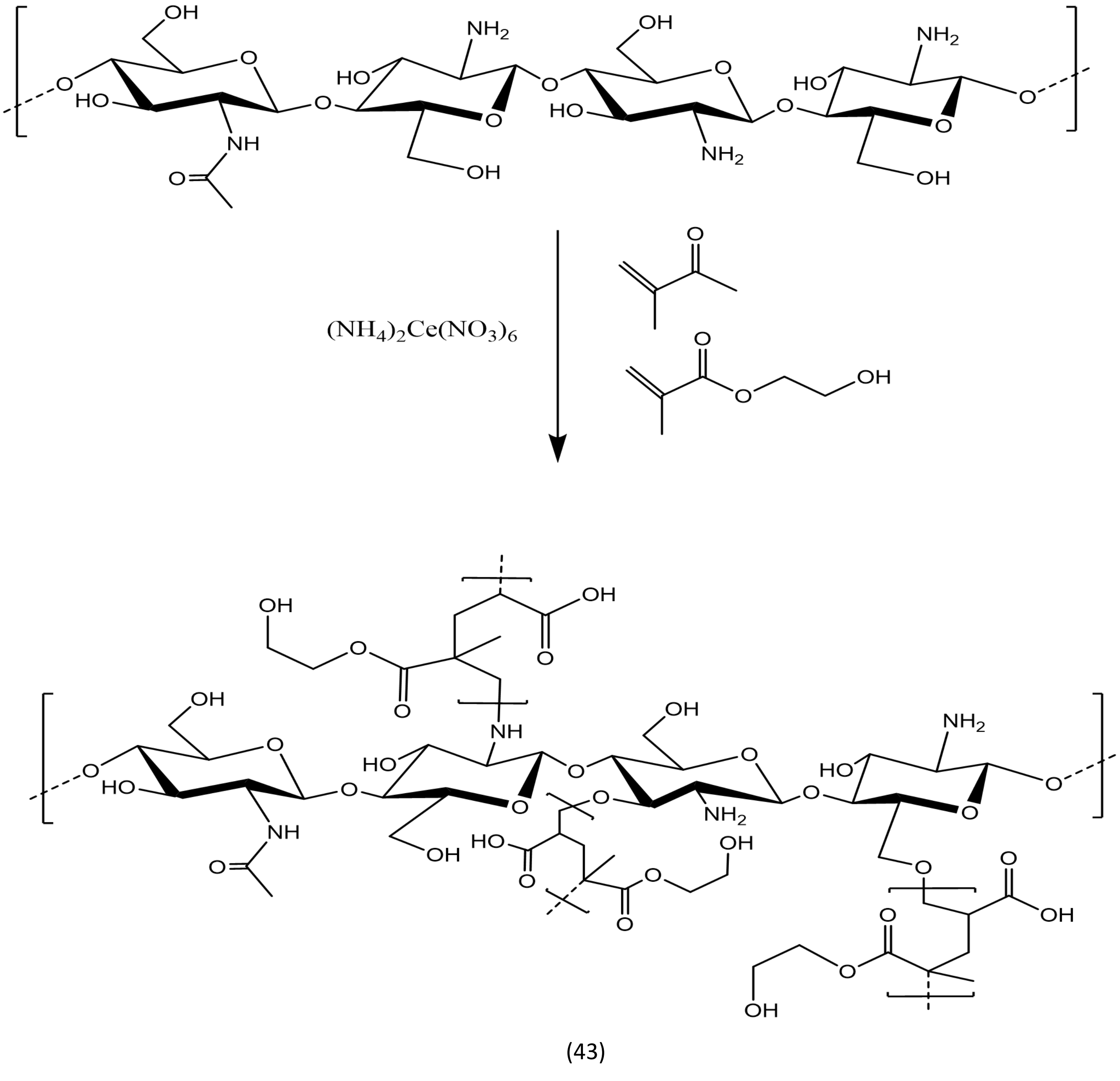
N. Barati *et. al.,* (2009) investigated the Preparation of uniform TiO2 nanostructure fifilm on 316L stainless steel by sol–gel dip coating.In this study , sol was prepared using tetra-n-butyle titant, ethanol and ethyl aceto acetate mixed together by stirring for 8hrs. The stainless steel (316L) was polished with alumina powder, ethanol and acetone. Dip coating method is followed at a various withdrawl speed. Drying follows either two paths, natural drying or in-solvent bath. XRD shows anatase phase formation. Different pH value sol's were prepared to form film, further analysed in AFM and SEM. This resulted as morphology of surface and structure of crystals (TiO_2) based on the calcination temperature and pH values of sol.

Magdy W. Sabaa *et. al.*, (2017) analyzed the synthesis, characterization and antimicrobial activity of Schiff base modified chitosan-graft-poly(acrylonitrile). In this study, they have synthesised chitosan-graft-poly(acyrlonitrile) (Ch-g-PAN) (40) by free radical polymerization of chitosan (38) with acrylonitrile (39) by using potassium per sulphate as initiator under nitrogen atmosphere. Further Ch-g-PAN treated with aldehyde which results schiff base modified chitosan-graft-poly(acyrlonitrile) (Ch-g-PAN I-V) (41).



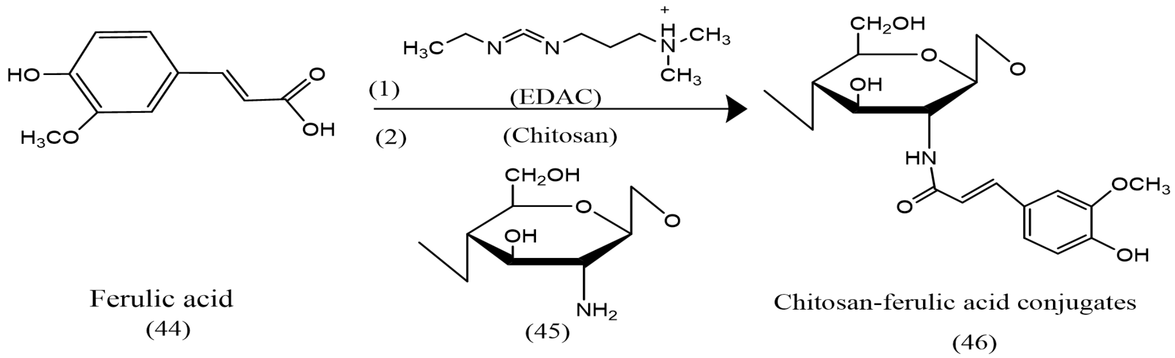
Yujia Diao *et. al., (2019)* studied Quercetin-grafted chitosan prepared by free radical grafting:characterization and evaluation of antioxidant and antibacterial properties. In this study they analysed chitosan in acetic acid and ethanol added H_2O_2 solution (contains ascorbic acid) and quercetin solution in different ratios kept in dark for 24hrs to synthesis quercetin grafted chitosan by Yujia Diao, auercetin increased the water solubility of chitosan and reduced the antibacterial activity.

K.S.C.R. dos Santos *et. al.*, (2006) studied the synthesis and characterization of membranes obtained by graft copolymerization of 2-hydroxyethyl methacrylate and acrylic acid onto chitosan. In this study, they have developed acrylic acid (AA) and 2-hydroxyethyl methacrylate (HEMA) grafted chitosan (43) by graft copolymerization using cerium ammonium nitrate as initiator on a petri dish. The chitosan (42) conjugate is suitable to use as drug delivery system on wound healing.

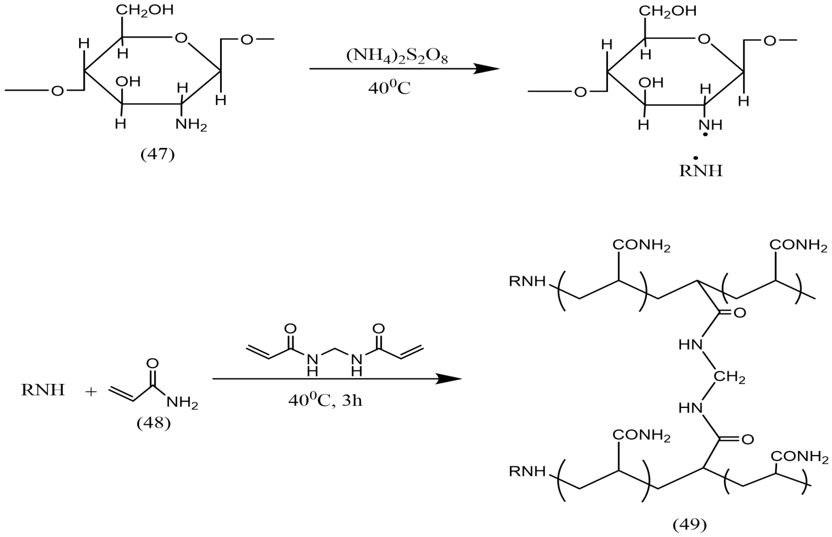


(43)

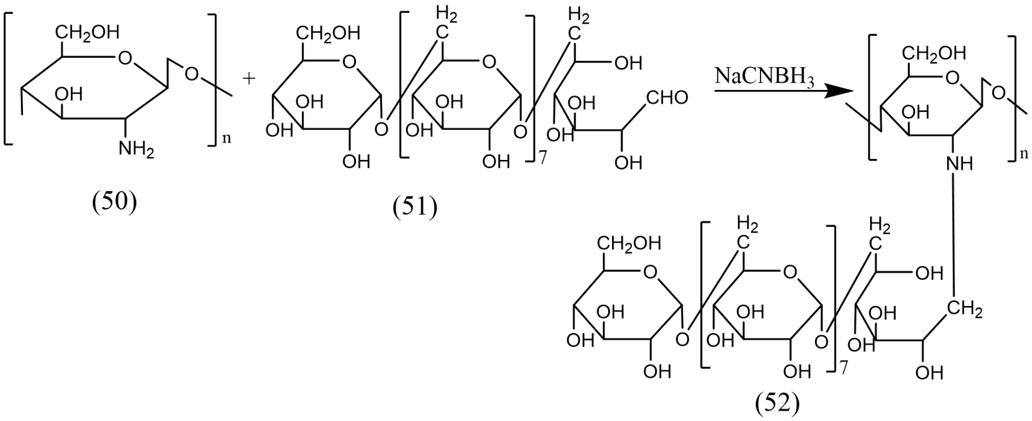
Chen Li *et. al.,* (2020) investigated the evaluation of chitosan-ferulic acid microcapsules for sustained drug delivery: Synthesis, characterizations, and release kinetics in vitro. In this study, they have synthsised chitosan-feulic acid conjugate (46) by carbodiimide mediated coupling reaction between feulic acid (44) and chitosan (45) solution under continuous flow of oxygen free nitrogen gas.



Guoqi Fu *et. al.*, (2007) studied he bovine serum albumin-imprinted polymer gels prepared by graft copolymerization of acrylamide on chitosan. In this study, they have prepared acrylamide chitosan conjugate (49) by graft copolymerization of N,N-methylenebisacrylamide (48) on chitosan (47) using ammonium persulfate as indicator. From the conjugate bovine serum albumin-imprinted polymer gels are prepared which increased the binding capacity of the gel.



Ugne Janciauskaite ***et. al.,*** **(2008)** studied the synthesis and properties of chitosan-N-dextran graft copolymers. In this study, they have developed chitosan-N-dextran polymer (52) by reductive amination of chitosan (50) and dextran (51), using sodium cyanoborohydride as initiator. The chitosan-N-dextran polymer could be used as surface conditioners.

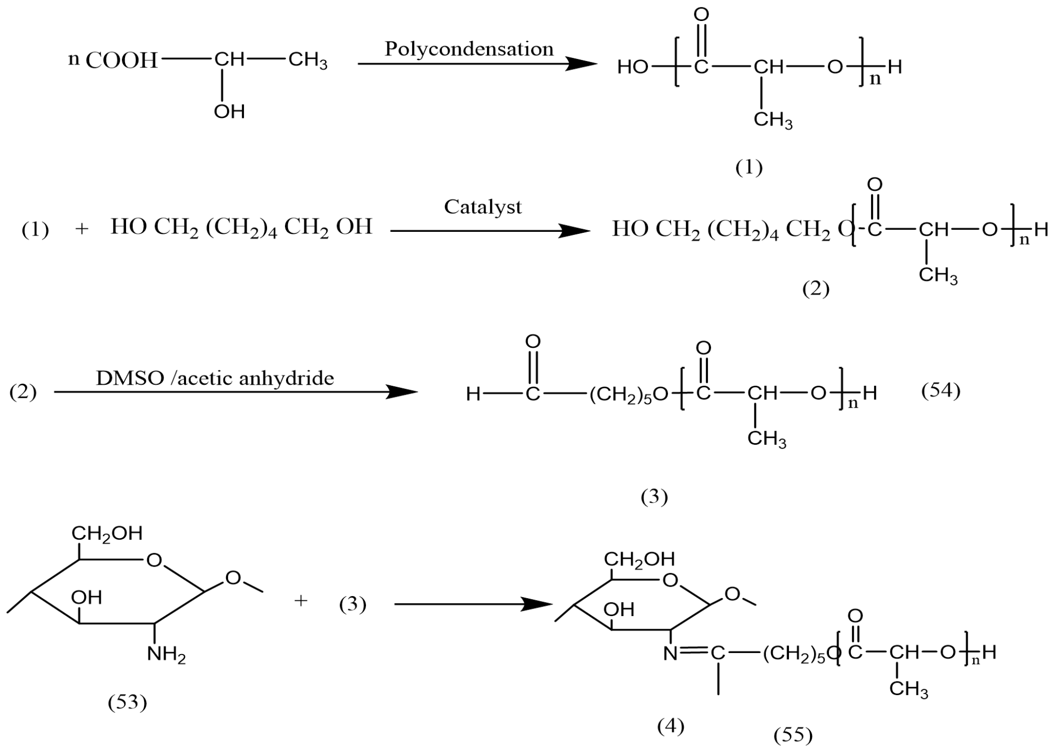


(50) (51) (52)

N Barathi ***et. al.,*** **(2009)** studiet the prparatio of uniform TiO_2 nanostructure film on 316 stainless steel by sol-gel dip coating. In this study, the sol of TiO_2 was prepared by dissolving 4 molar percent of tetra-n-butyl titan in ethanol and ethyl aceto acetate. The solution was kept in stirrer for 8hrs. Deionized water was added for hydrolysis. Finally the solution was aged for 24hrs to complete the reactions. Then coated on 316L stainless steel via dip coating method. Different variant sol such as pH, withdrawl,speed, calcination temperature have been used and each varied sol was coated on 316L SS. The coated steels were analysed uder SEM. Optical microscopy and XRD. In acidic region, the TiO_2 crystal size decreased by decreasing pH values of sol.

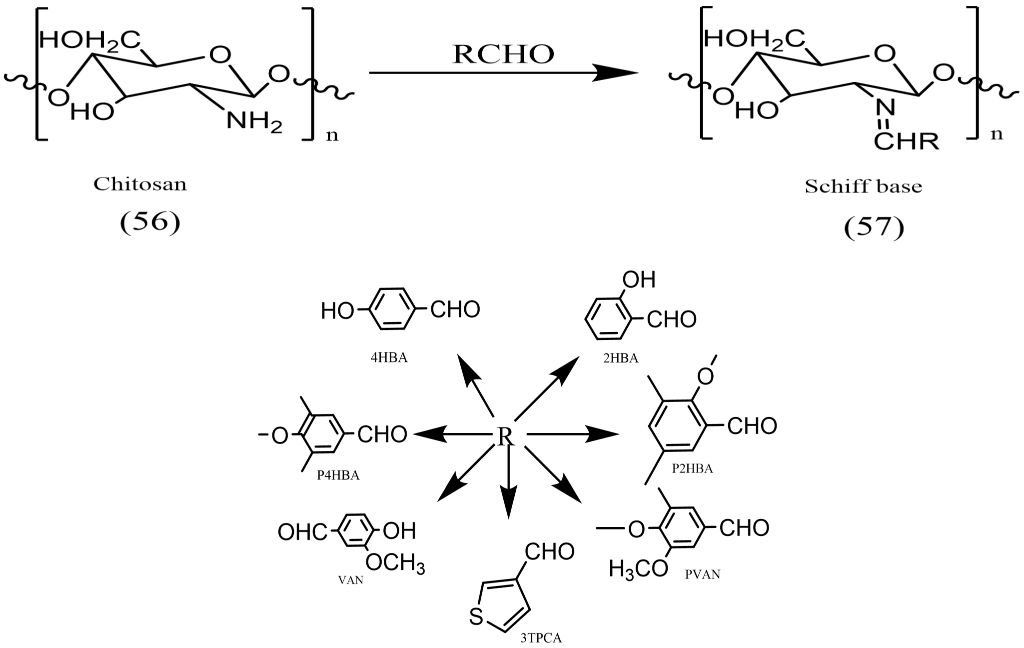
Meifang Huang ***et. al.,*** **(2005)** investigated the study of graft copolymerization of N-maleamic acid -chitosan and butyl acrylate by γ-ray irradiation. In this study, they have synthesised N-maleamic acid-chitosan-g-poly butyl acrylate through graft polymerization of previously prepared N-maleamic acid -chitosan and butyl acrylate kept in ^{60}CO γ-ray chamber.

Fanglian Yao *et. al.*, (2003) analysed the synthesis and characterization of chitosan grafted oligo(L-lactic acid). In this study, chitosan grafted oligo(L-lactic acid (55) is prepared by graft polymerization of L-lactic acid and chitosan (53) solution under nitrogen atmosphere using sodium cyanoborohydride as initiator.



Mohammadreza Foruzanmehr *et. al.,* (2013) studied the Nano-structure TiO_2 film coating on 316L stainless steel via sol-gel technique for blood compatibility improvement . In this study, they have developed a TiO_2 film on 316L stainless steel by primarily preparing TiO_2 sol with titanium tetra iso peroxide ethanol water in the ratio of 1:0.75:83, reflexed in 80C for 12 hours under stirring and kept 48 hrs in room temperature. Secondly they have dip coated 316L stainless steel plants into sol upto 5 layers, dried at 100C, annealed at 50C in nitrogen purged furnace for 10 min. It exhibit the TiO_2 (nano) coating on 316L stainless steel elevates blood compatibility.

Hatice Karner *et. al.,* (2015) studied the synthesis, characterization and thermal decomposition of Schiff base (57) polymers containing chitosan unit. In this study, a series of chitosan (56) Schiff base polymer by copolymerization of Schiff bases such as 4-hydroxy benzaldehyde (4HBA), vanillin (VAN), 3-thiophenecarboxaldehyde (3TPCA) and chitosan solution (dissolved in acetic acid).



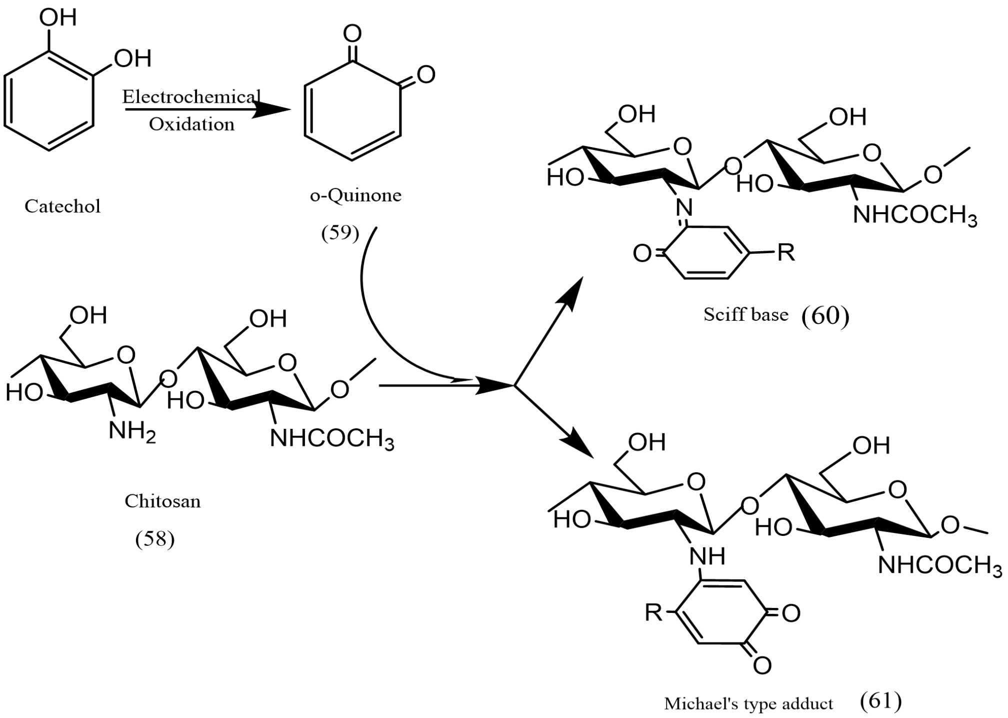
Chitosan (56)

Schiff base (57)

Niladri Sekhar Chatterjee *et. al.*, (2015) analysed the vanillic acid and coumaric acid grafted chitosan derivatives: improved grafting ratio and potential application in functional food. In this study, they have synthesised a series of chitosan-phenolic acid conjugates by free radical medicated grafting technique on chitosan solution and series of phenolic acid such as gallic acid, ferulic acid, vanillic acid and coumaric acid.

Fanglin Yao *et. al.*, (2003) investigated a study on cytocompatible poly(chitosan-g-L-lactic acid). In this study, they have developed chitosan-Lactic acid grafted polymer without any initiator by dissolving chitosan(CS) powder in aqueous solution of L-lactic acid. The sample is treated with chloroform and methanol to remove unreacted L-lactic acid.

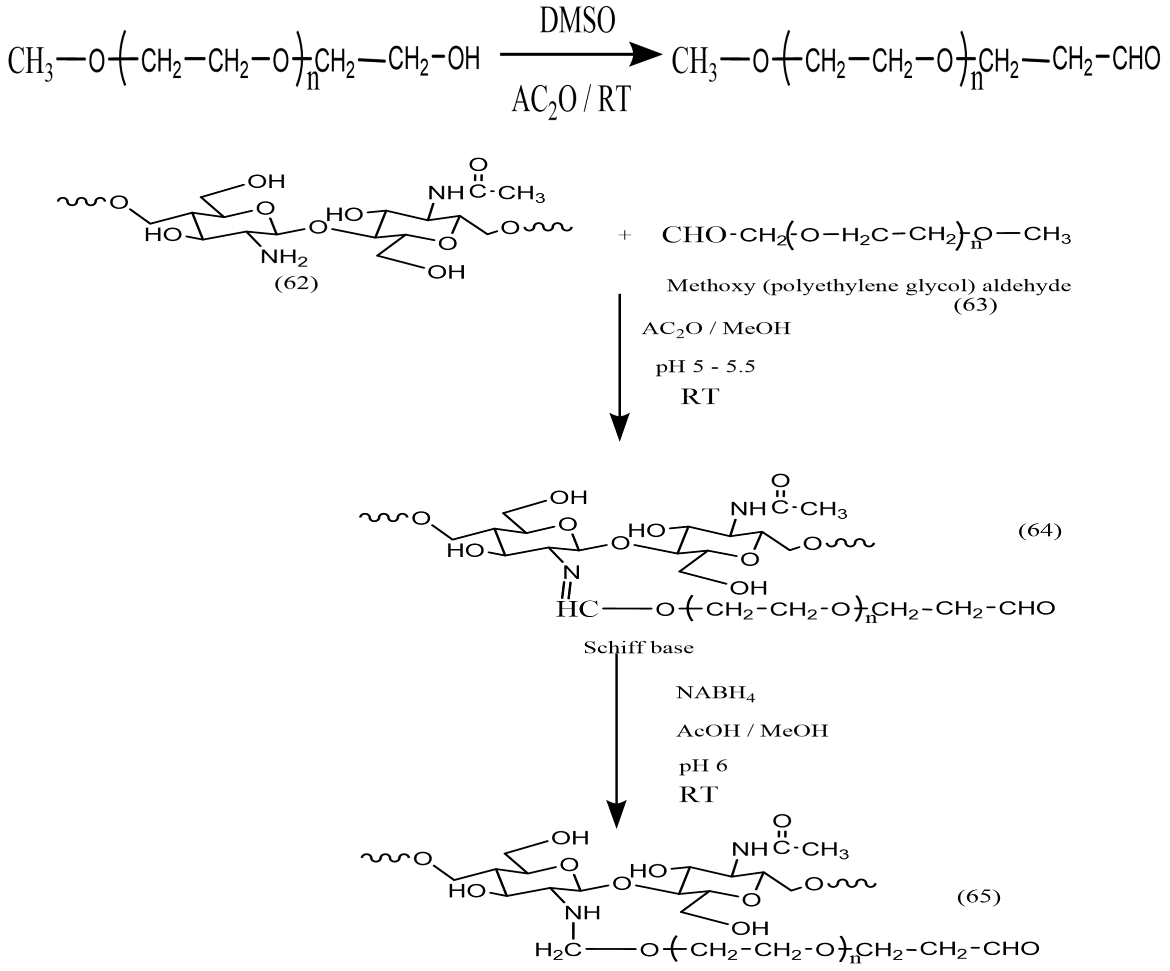
Jun Liu *et. al.,* (2017) studied the synthesis, characterization, bioactivity and potential application of phenolic acid grafted chitosan – a review. In this study, they have reviewed the synthesis of phenolic-g-chitosan by grafting of catecholic reacants (59) on to chitosan(58) film using electro chemical method via Michael type addition (61) and Schiff base (60) reactions.



Tao Sun *et. al.*, (2003) studied the graft copolymerization of methacrylic acid onto carboxymethyl chitosan. In this study, they have investigated the methacrylic acid on carboxymethyl chitosan (CMCTS) by graft copolymerization using ammonium persulfate as initiator to the reaction between methacrylic acid and carboxymethyl chitosan.

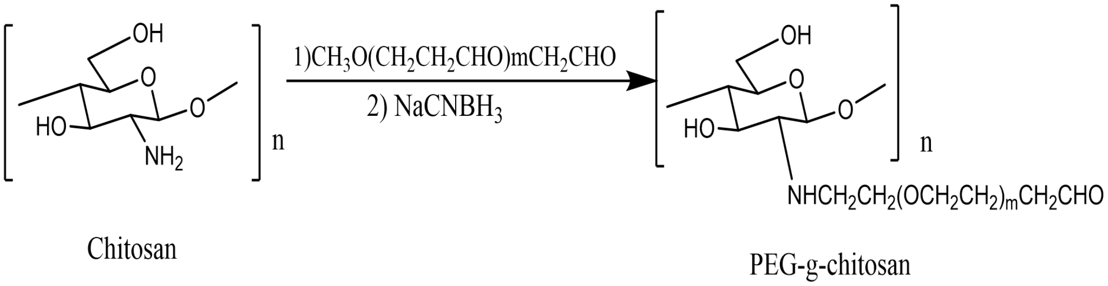
Anna Pawlik *et. al.,* (2007) studied the Fabrication and characterization of electrophoretically deposited chitosanhydroxyapatite composite coatings on anodic titanium dioxide layers. In this study, they prepared anatase TiO_2 and suspension with chitosan and hydroxyapatite using acetic acid and ethanol as solvent then ultrasoniated for 1 hour. They did electrophorectic deposition of TiO_2 chitosan hydroxyapaptite on 316L SS by using anodic nanoporous titanium di oxide as cathode and 316L SS as anode.

A.M.Abdul-Mohsen ***et. al.,*** **(2012)** studied the biomedical textiles through multifunctioalization of cotton fabrics using innovative methoxypolyethylene glucol-N-chitosan graft copolymer. In this study, they have prepared methoxy polyethylene glycol-N-chitosan graft copolymer (65) by addition of chitosan (62) and methoxy (polyethylene glycol) aldehyde (63) to Schiff base (64), then reductive alkylation with sodium borohydride.



A. Silva-Dias ***et. al.,*** **(2013)** analysed the Anti-biofilm activity of low-molecular weight chitosan hydrogel against Candida species.In this study, they have investigated the anti-bioflim activity of chitosan hydrogel on candida species by suspending low molecular of weight chitosan coated with polystyrene plates into yeast for 24 hours. The bio-flim formation is measured using reduction array to measure the biomass of bio-flim and bio-flim cellular activity. It proves that chitosan have the ability to reduce the bio-flim activity.

Narayan Bhattarai *et. al.,* (2004) studied PEG-grafted chitosan as an injectable thermoreversible hydrogel. In this study, they have synthesised PEG(polyethylene glycol)- grafted chitosan (67) by the reaction between PEG-aldehyde and chitosan (66) using cyanoborohydride as reducing agent.

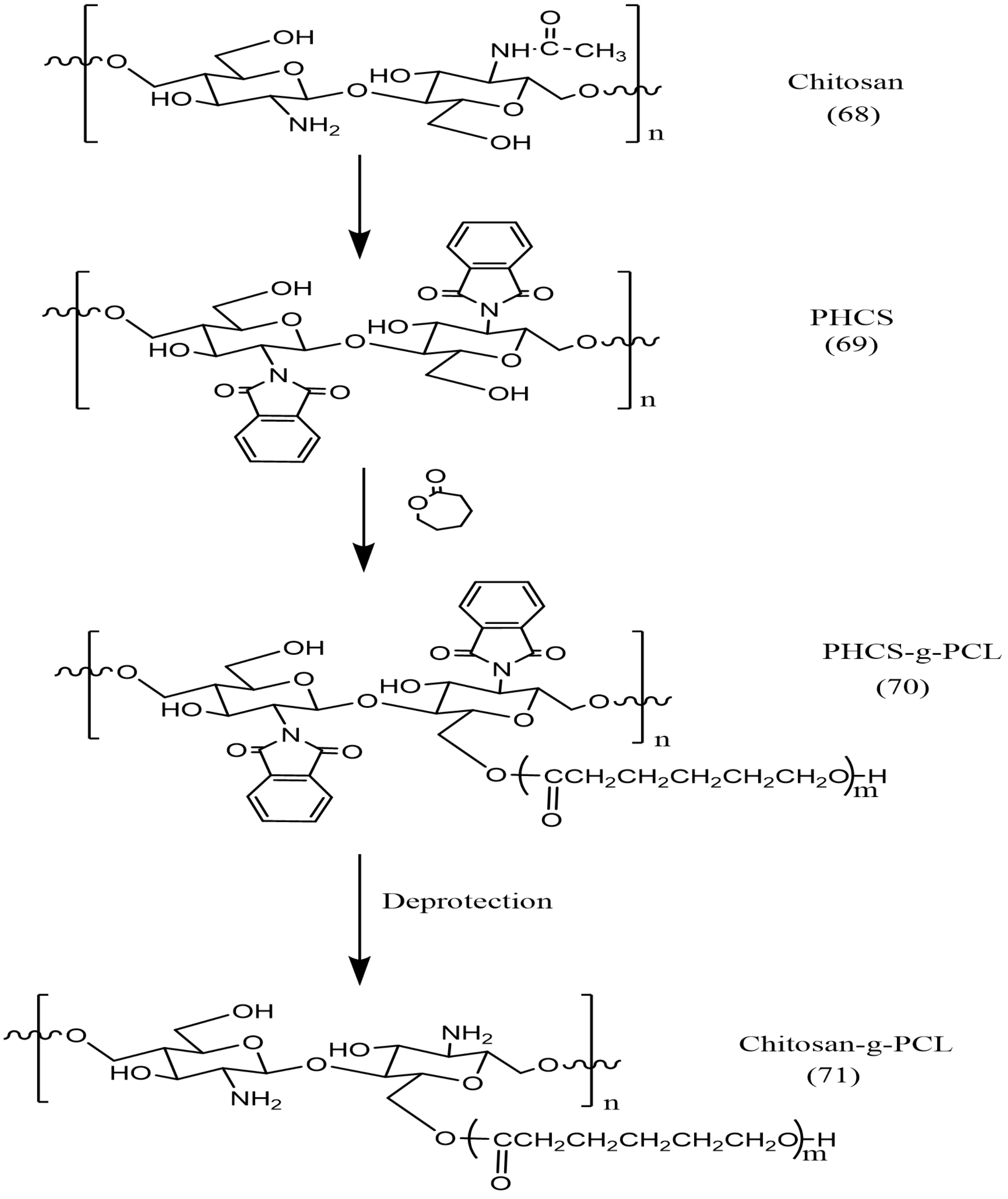


(67)

A. Balamurugan *et. al.,* (2007) analysed the Electrochemical and structural characterisation of zirconia reinforced hydroxyapatite bioceramic sol–gel coatings on surgical grade 316L SS for biomedical applications. In this study they have developed zirconia reniforced hydroxyapatite coated on 316L SS by sol gel dipping method. Sol gel prepared by stirring zircornium peroxide with triethyl phosphate (dissolved in anhydrous ethanol) and calcium nitrate (dissolved in anhydrous ethanol) stirred to make Ca-P solution as precursor using yttrium acetate as stabilizer.

Sam John *et. al.,* (2016) studied Inibition of Mild Steel Corrosion using Chitosan-Polyvinyl Alcohol Nanocomposite Films by sol gel Method: An Environmentally Friendly Approach.In this study, chitosan grafted polyvinyl alcohol films were developed by sonificate the mixture of polyvinyl alcohol and chitosan in 100ml of 1% acetic acid. The homogeneous solution is further stirred for 12 hrs with 70°C heating. Then stirring is continued for another 12hrs at room temperature. It results PVA grafted chitosan blend which on further heating the concentration of polymeric blend increases resulted a sol of PVA-g-chitosan. The sol was coated on mild steel kept in room temperature overnight for drying. The surface morphology of the sample was analysed under SEM and corrosion parameters were determined by Tafel polarization

Li Liu *et. al.,* (2006) studied the self-catalysis of phthaloylchitosan for graft copolymerization of ε-caprolactone with chitosan. In this study, they have heated chitosan (68) with phthalic anhydride (PHCS) (69) to produce PHCS-g-polycaprolactone (PHCS-g-PCL) (70), which is further graft polymerised with caprolactone under nitrogen atmosphere gave phthalic anhydride chitosan (Chitosan-g-PHCS) (71)



Zetty Azalea Sutirman *et. al.,* (2017) analysed the ammonium persufate-initiated graft copolymerization of methacrylamide onto chitosan: synthesis, characterization and optimization. In this study, they have used graft copolymerization method in methacrylamide and chitosan solution by free radical mechanism using ammonium persulfate (APS) as initiator .

Grafting at O-H groups of chitosan

$\mathrm{S_2O_8^{2-}} \longrightarrow \mathrm{SO_4^{\cdot -}}$ initiation

$\mathrm{SO_4^{\cdot -}} + \mathrm{ChOH} \longrightarrow \mathrm{Ch\dot{O}}$

$\mathrm{Ch\dot{O}} + \mathrm{M} \longrightarrow \mathrm{ChOM^{\cdot}}$ propagation

$\mathrm{ChO\dot{M}} + \mathrm{M_n} \longrightarrow \mathrm{ChOM\dot{M}_n}$

$\mathrm{ChOM\dot{M}_n} + \mathrm{ChOM\dot{M}_n} \longrightarrow$ graft copolymer termination

Grafting at N-H groups of chitosan

$\mathrm{S_2O_8^{2-}} \longrightarrow \mathrm{SO_4^{\cdot -}}$ initiation

$\mathrm{SO_4^{\cdot -}} + \mathrm{ChNH} \longrightarrow \mathrm{Ch\dot{N}}$

$\mathrm{ChN^{\cdot}} + \mathrm{M} \longrightarrow \mathrm{ChNM^{\cdot}}$ propagation

$\mathrm{ChN\dot{M}} + \mathrm{M_n} \longrightarrow \mathrm{ChNM\dot{M}_n}$

$\mathrm{ChNM\dot{M}_n} + \mathrm{ChNM\dot{M}_n} \longrightarrow$ graft copolymer termination

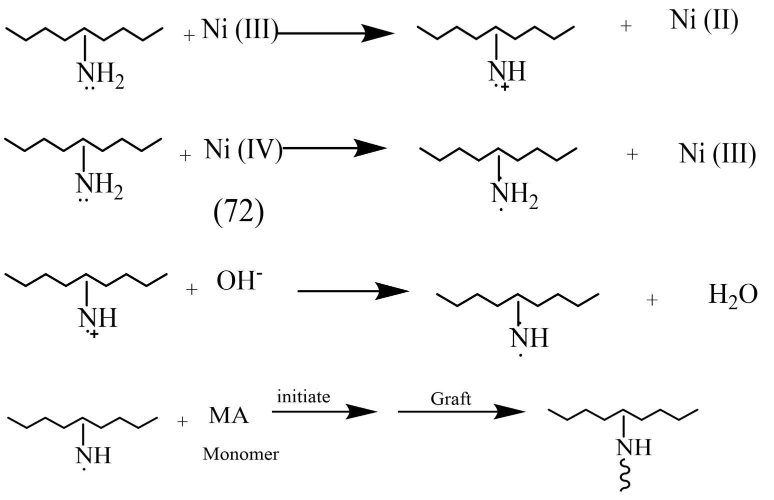
$\mathrm{\dot{M}} + \mathrm{M} \longrightarrow \mathrm{MM^{\cdot}}$

$\mathrm{\dot{M}_{n-1}} + \mathrm{M} \longrightarrow \mathrm{M^{\cdot}}$

$\mathrm{ChOH_2} / \mathrm{ChNH_2} + \mathrm{M^{\cdot}} \longrightarrow \mathrm{ChO\dot{H}} / \mathrm{ChN\dot{H}} + \mathrm{M_n}$ homopolymer

where Ch represents chitosan, M for methacrylamide

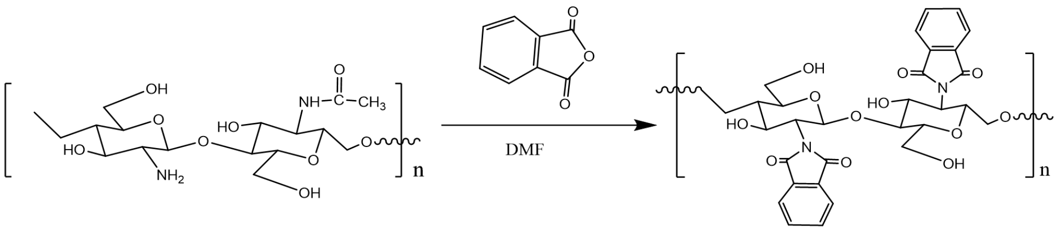
Yinghai Liu *et. al.*, (2014) studied the graft copolymerization of methyl acrylate onto chitosan initiated by potassium diperiodatonickelate (IV). In this study, they have synthesised methyl acrylate chitosan (75) derivative by graft polymerization of chitosan (73) acidic solution and methyl acrylate (74) using potassium diperiodatonickelate (IV) (72) as initiator.



(72) (73) (74) (75)

Li Liu *et. al.*, (2004) studied the synthesis and characterization of chitosan-graft-polycaprolactone copolymers. In this study, they have investigated the synthesis of chitosan-graft-polycaprolactone copolymer (78) through a protin-graft deprotection route with chitosan (76) and polycaprolactone (77) using phthaloyl chitosan as intermediate, under nitrogen atmosphere using DMF as solvent.

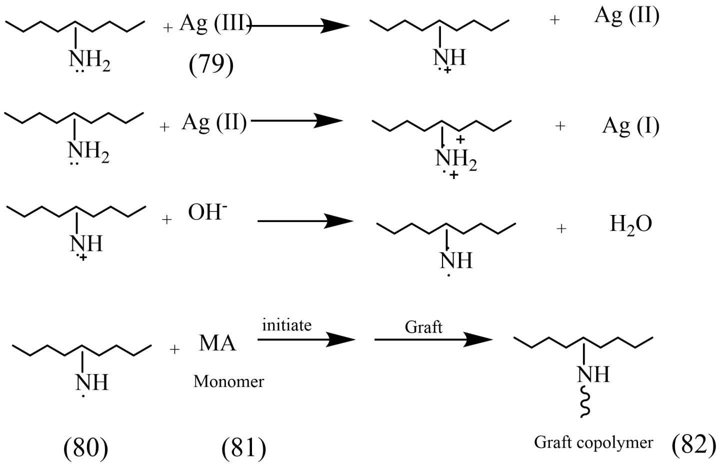
(77)



(76) (78)

Hatico Nilay Hasipoglu *et. al.,* (2007) studied the preparation and characterization of maleic acid grafted chitosan. In this study, they have synthesised maleic acid grafted chitosan from maleic anhydride and acidic solution of chitosan under nitrogen atmosphere using cerium ammonium nitrate as initiator, acetone is used to precipitate the product.

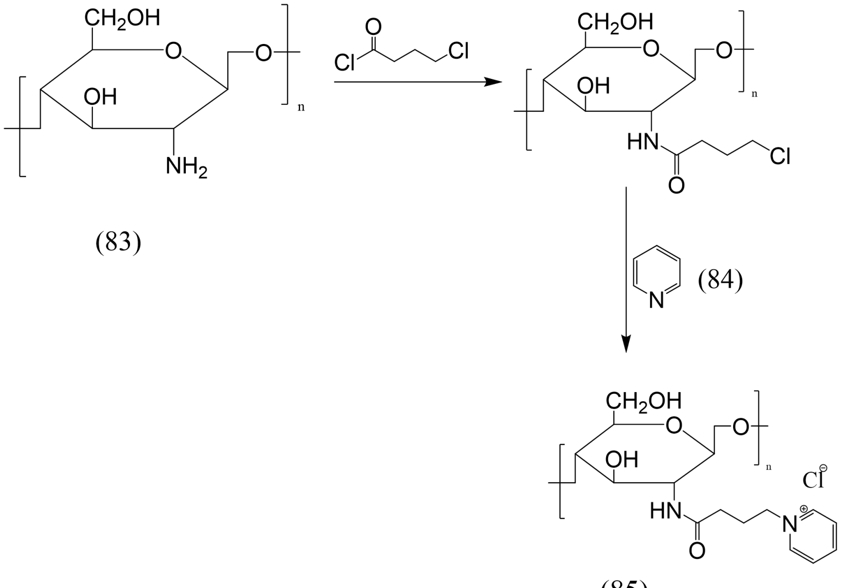
Zhenghao Liu *et. al.,* (2005) analysed the graft copolymerization of methyl acrylate onto chitosan initiated by potassium diperiodatoargentate (III). In this study, they have developed methyl acrylate chitosan (82) through graft copolymerization between methyl acrylate (81) and chitosan (80) solution using potassium diperiodatoargentata (III) (79) as initiator.



Yingjun Jing *et. al.,* (2019) analysed the Maintenance of the antioxidant capacity of fresh-cut pineapple by procyanidin-grafted chitosan. In this study, procyanidin- grafted chitosan used as an edible coatings in pineapple. PCCS prepared by free radical medicated procedure between chitosan and procyanidin. Chitosan dissolved in acetic acid and H_2O_2 solution then agitated for 30mins. Procyandin added to the solution, pH was maintained using NaOH solution. PCCB increased the productivity on vitamin C and polyphenols in fresh cut pineapples.

Jun Liu *et. al.,* (2014) analysed Effect of Protocatechuic Acid-Grafted-Chitosan Coating on the Postharvest Quality of Pleurotus eryngii. In this study, protocatechnic acid grafted chitosan was prepared using carbodiimide hydrochloride, N-hydroxy succinimide as catalyst in a reaction between chitosan and protocachnic acid using 2-(N-morpholinol) ethane sulfonic acid as a buffer solution via coupling method. The resultant could be used as an antioxidant in food.

Ruixiu Jia *et. al.,* (2016) studied Pyridine-grafted chitosan derivative as an antifungal agent In this study they have analysed pyridine chitosan (85) an antifungal agent synthesised via nucleophilic substitution between N-chlorobutyryl chitosan and pyridine. Chitosan (83) dissolved in N-methyl pyrrolidone and 4-chlorobutyryl dissolved N-methyl pyrrolidine solution was stirred, washed with methone and diethyl ether. The obtained N-chlorobutyryl chitosan stirred with pyridine and washed with same as above. Pyridine chitosan showed positive result as an anti-fugal agent against B-cinera and F-fulva.



(83)

(84)

(85)

Naruedee Bandatang *et. al.,* (2021) analysed Antimicrobial electrospun nanofiber mats of NaOH-hydrolyzed chitosan (HCS)/PVP/PVA incorporated with in-situ synthesized AgNPs: Fabrication, characterization, and antibacterial activity. In this study, molecular weight of chitosan was reduced by NaOH hydrolysis. The NaOH hydrolysed chitosan prepared by .dissolving chitosan in 50% NaOH and stirred for 24hrs at 90^{o}C. Then washed with deionized water, neutralized with ACOH. Further hydrolysed chitosan used to synthesized in situ silver nano particles chitosan via electrospinning method. It exhibit moderate bactericidal activity on the nano fibres.

Muhammad Atiq Ur Rehman *et. al.,* (2018) studied the Electrophoretic deposition of chitosan/gelatin/bioactive glass composite coatings on 316L stainless steel: A design of experiment study. In this study chitosan, gelatin and copper doped bioactive glass composites are coated on 316L stainless steel by electrophoretic deposition. Different composition of suspensions are made using acetic acid, ethanol. 316L SS was cut into 3cm x 1.5cm and cleansed with acetone and ethanol. When current is passed the suspension is deposited on 316L SS. Taguchi DOE, SEM resulted that the coatings are maximum when deposition voltage of 30V, time of 5 mins and chitosan gelatin ratio of 50:50. Further it is analysed under tape test and bending test, which exhibited positive result.

Sheng Meng *et. al.,* (2009) analysed The effect of a layer-by-layer chitosan–heparin coating on the endothelialization and coagulation properties of a coronary stent system. In this study coronary stent were prepared from 316L SS. The cleansing process were done by using alcohol and ethanol solution of 3-aminopropyltriethoxysilane. The stent were primarily dipped in heparin sodium salt solution then into chitosan solution. The procedure was repeated to obtain several layers.

MATERIALS METHODS &

3. MATERIALS AND METHODS

3.1 GENERAL

- Low molecular weight chitosan (extra pure) was purchased from Loba chemicals.

- 1% acetic acid is prepared by adding 0.1 ml of concentrated acetic acid to 0.9 ml of water.

- O.1 M Ammoniumpersulphate solution prepared by dissolving 0.2281g of ammoniumpersulphate in 10 ml of water.

- 316 L stainless steel is cut into 1mm×1mm dimension and 60mm×20mm dimension plates with 0.5 mm thickness.

- 120 gsm Grit sheet is used to polish 316L stainless steel.

- The Fourier transform infrared (FTIR) spectrum were recorded by ATR technique in a SHIMADZU PRESTIGE 20 FT-IRSpectrometer. Absorption frequencies were quoted in receiprocal centimeter.

- The Thermal gravimetric analysis done by STA module with no reference from 23°C to 1000° C.

- The Scanning electron microscope was performed using ZEISS sem analyser with 1000 magnification.

3.2 PREPARATION OF ACID GRAFTED CHITOSAN

3.2.1 Preparation of Mandalic acid grafted Chitosan:

0.2509 g of low molecular weight chitosan was dissolved in 3 ml of 1% acetic acid (solution A) 0.0258 g of mandalic acid was dissolved in 6 ml of 0.1 M ammonium per sulphate (solution B). Solution B was added to solution A and the mixture was stirred for 24 hours. After formation of polymer,the contents were treated with acetone. The solid obtained was filtered and dried. 0.39 g yield was obtained.

3.2.2 Preparation of p-Coumaric acid grafted Chitosan:

0.2509 g of low molecular weight chitosan was dissolved in 3 ml of 1% acetic acid (solution A) 0.0258 g of p-coumaric acid was dissolved in 6 ml of 0.1 M ammonium per sulphate (solution B). Solution B was added to solution A and the mixture was stirred for 24 hours. After formation of polymer,the contents were treated with acetone. The solid obtained was filtered and dried. 0.33 g yield was obtained.

3.2.3 Preparation of Dipicolinic acid grafted Chitosan:

0.2509 g of low molecular weight chitosan was dissolved in 3 ml of 1% acetic acid (solution A) 0.0258 g of dipicolinic acid was dissolved in 6 ml of 0.1 M ammonium per sulphate (solution B). Solution B was added to solution A and the mixture was stirred for 24 hours. After formation of polymer,the contents were treated with acetone. The solid obtained was filtered and dried. 0.37 g yield was obtained.

3.2.4 Coating of acid grafted chitosan polymer on 316L stainless steel:

0.05 g of acid grafted chitosan (MA-g-CH, PCA-g-CH or DPA-g-CH) was 50 ml dispersed in 1% aqueous acetic acid solution using ultra sonication till it homogenized. To the homogenous solution 0.5 g of glycerol was added and kept in the stirrer for 6 hours. 316 L stainless steel sample were cut into 10mm× 10mm dimension. The samples were polished from 120 grit silicon carbid paper and washed with distilled water. To the prepared solution, the samples were dipped for 5 minutes with the same withdrawal speed. The coated samples were dried and kept in a fume hood for 24 hours. The same procedure was carried out with pH variant by maintaing the pH using NaOH and Conc. HCl.


RESULT and discussion

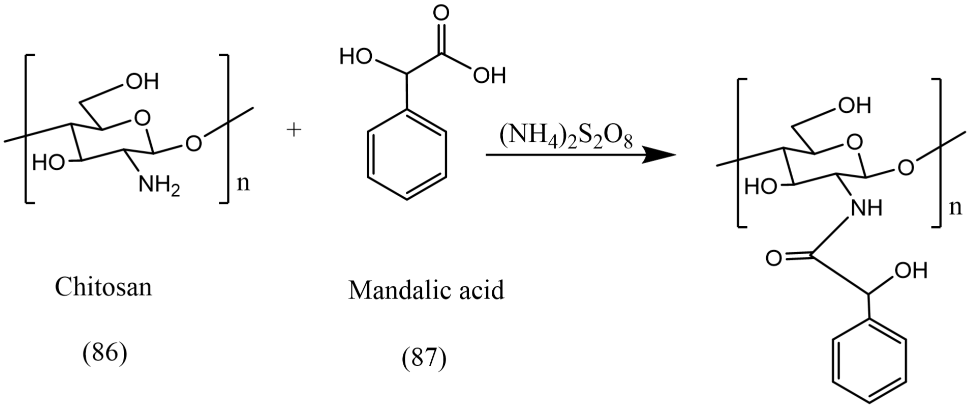
4. RESULTS AND DISCUSSION

4.1 The reaction of acid with chitosan has been reported by few authors with the different types of products such as ferulic acid grafted chitosan **(Chen Li *et. al.,* 2017)** and vanilic acid grafted chitosan **(K.V. Vishnu *et. al.,* 2017)**

In the present work mandalic acid, p-coumaric acid and dipicolinic acid is treated with chitosan to obtain highly grafted chitosan polymer. Further, the polymers are coated on to 316L stainless steel. The 316L stainless steel is used in dental procedures and implants in human body due to low of carbon content.**(D.W. Hoppner and V. Chandrasekaran, 1993)**

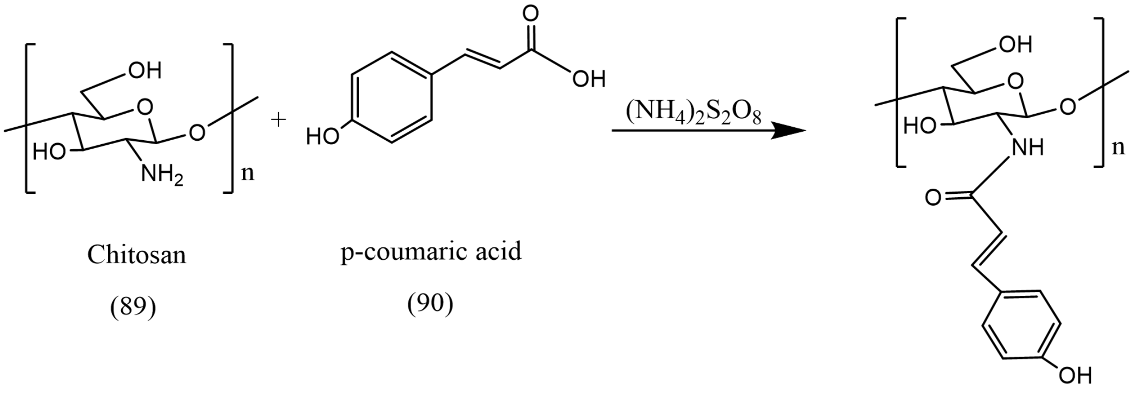
The reaction was carried out by stirring the mixture of acid with 0.1 M ammonium persulphate solution and chitosan with 1% acetic acid for 24 hours, without heat. After formation of polymer,the contents were treated with acetone. The solid obtained was filtered and dried. The yield of the products were given in the **Table – I**. The sol gels of the products were made by dissolving the products in 1% acetic acid using ultra sonication and stirred with glycerol for 6 hours. Previously, cured 316L stainless steel coated with prepared sol gels.

Scheme I



MA-g-CH – Mandalic acid grafted chitosan

Scheme II



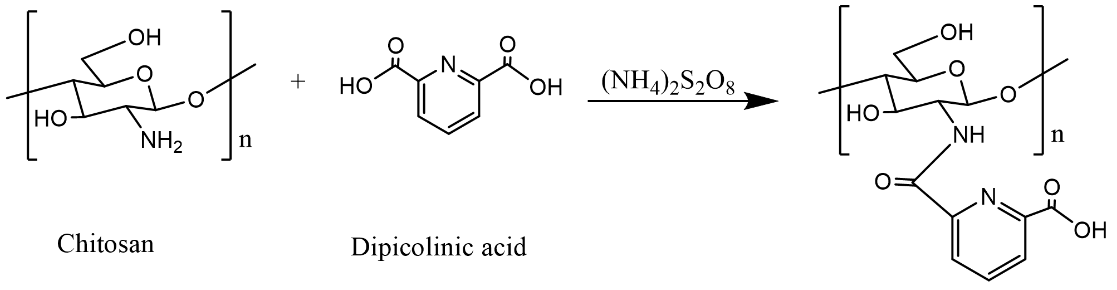
Chitosan (89)

p-coumaric acid (90)

PCA-g-CH (91)

PCA-g-CH – p-coumaric acid grafted chitosan

Scheme III



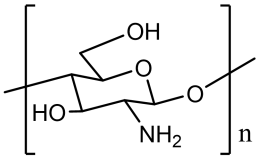
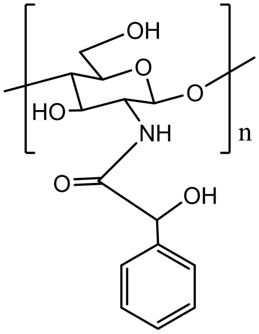
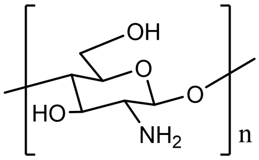
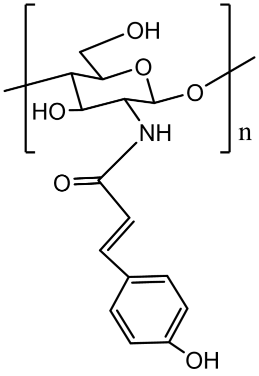
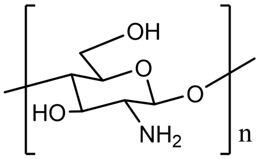
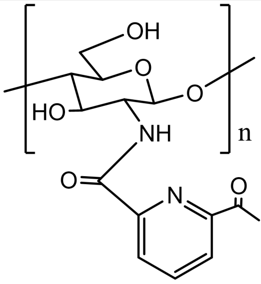
Chitosan (92)

Dipicolinic acid (93)

DPA-g-CH (94)

DPA-g-CH – Dipicolinic acid grafted chitosan

Table I: The yields of Product Formed

REACTANT	PRODUCT	CATALYST	YIELD
 (86)	 (88)	$(NH_4)_2S_2O_8$	99%
 (89)	 (91)	$(NH_4)_2S_2O_8$	96%
 (92)	 (94)	$(NH_4)_2S_2O_8$	98%

4.2 CHARACTERIZATION OF THE SYNTHESIZED COMPOUND

The synthesized polymers are characterized using FTIR, thermal stability was determined by TGA, solubility test and the surface morphologies of the coated 316L stainless steel was analyzed using SEM analysis.

4.2.1 FT-IR ANALYSIS

The IR spectrum of MA-g-CH, PCA-g-CH and DPA-g-CH are shown in figure 1, figure 2 and figure 3 respectively.

4.2.1.a Mandalic acid grafted chitosan

The FT-IR spectrum of mandalic acid grafted chitosan **(figure 1)** shows that the compound have a sharp band in 3778 cm^{-1} corresponding to –OH stretching in chitosan. A peak observed in 3209 cm^{-1} indicates NH stretching. In addition, the stretching vibration of NH-C=O was observed at 1629 cm^{-1}(amide I). A peak at 1402 cm^{-1} indicates OH bending of alcohol and 1747 cm^{-1} results of C=O stretching. A peak 1112 cm^{-1} denotes C-NH stretching vibration of the amide group.

4.2.1.b p-coumaric acid grafted chitosan

The FT-IR spectrum of p-coumaric acid grafted chitosan **(figure 2)** shows that the compound have a broad band in 3462 cm^{-1} corresponding to –OH stretching in chitosan. In addition, the stretching vibration of NH-C=O was observed at 1618 cm^{-1} (amide I). A peak at 1402 cm^{-1} indicates OH bending of alcoholic group. A peak 1114 cm^{-1} denotes C-NH stretching vibration of the amide group.

4.2.1.c Dipicolinic acid grafted chitosan

The FT-IR spectrum of dipicolinic acid grafted chitosan **(figure 3)** shows that the compound have a broad band in 3217 cm^{-1} corresponding to –NH stretching in chitosan. In addition, the stretching vibration of NH-C=O was observed at 1618 cm^{-1} (amide I). A peak at 1402 cm^{-1} indicates OH bending of alcohol. A peak 1112 cm^{-1} denotes C-NH stretching vibration of the amide group.

FIGURE 1: FT-IR of mandalic acid grafted chitosan

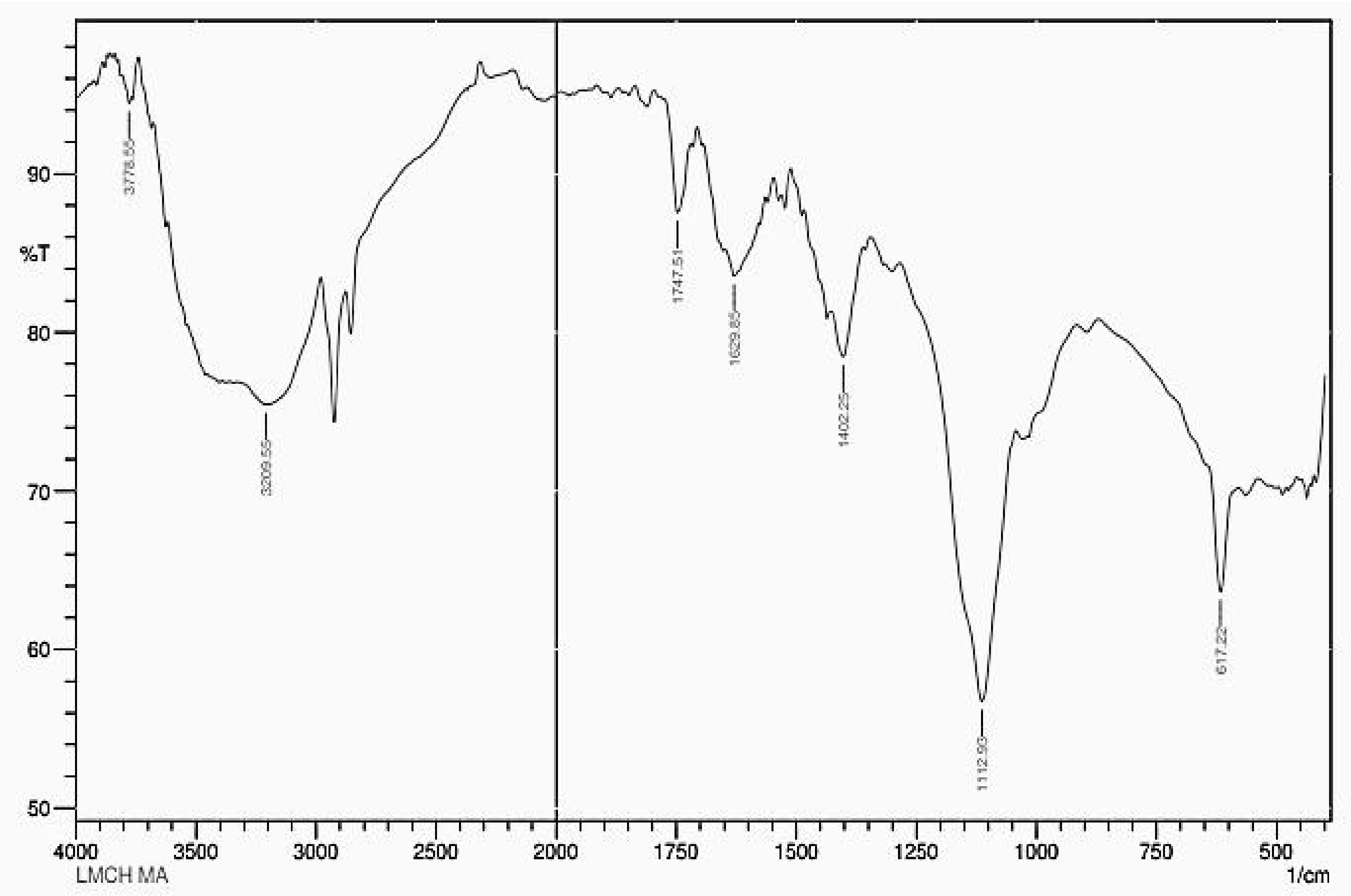


FIGURE 2:FT-IR of p-coumaric acid grafted chitosan

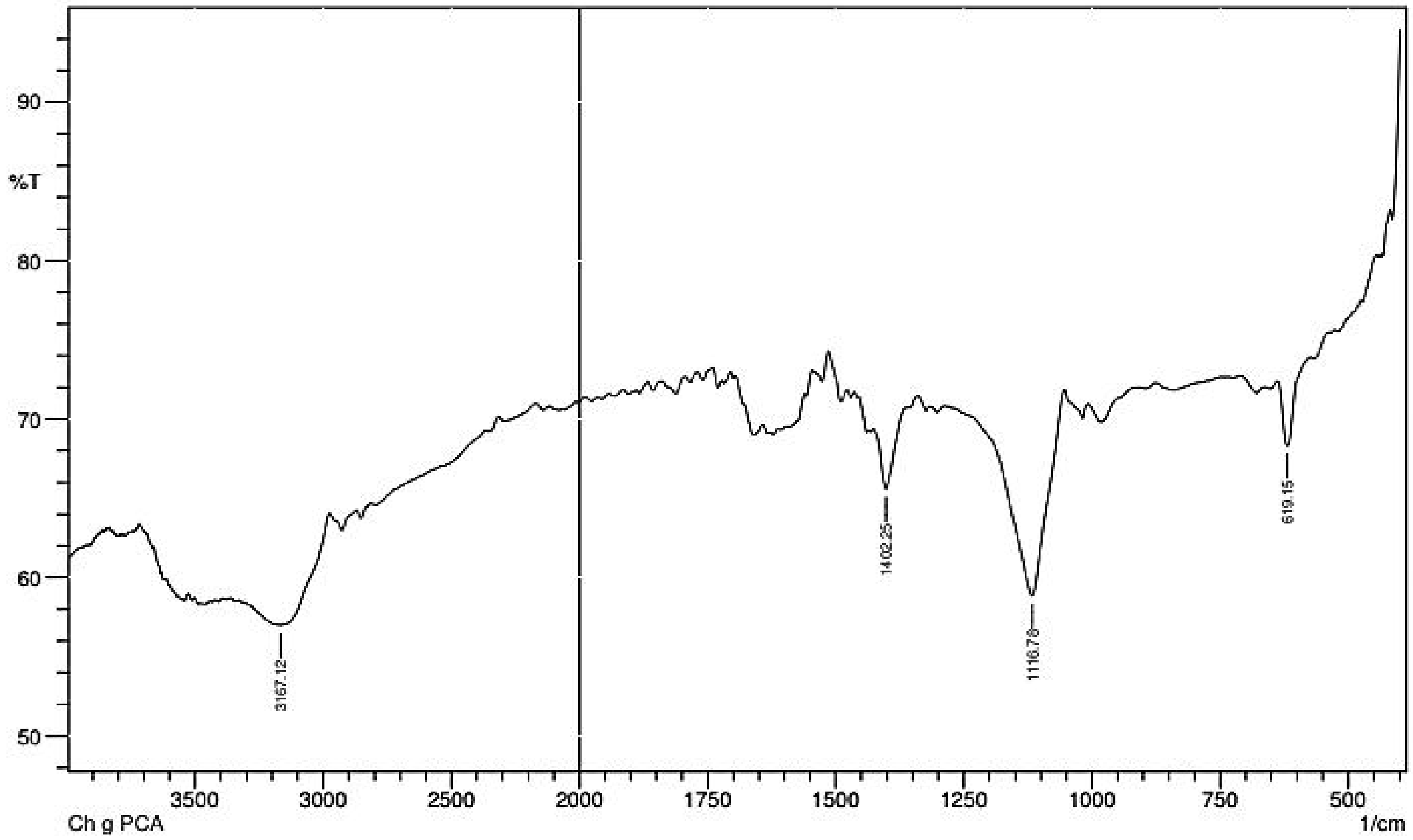
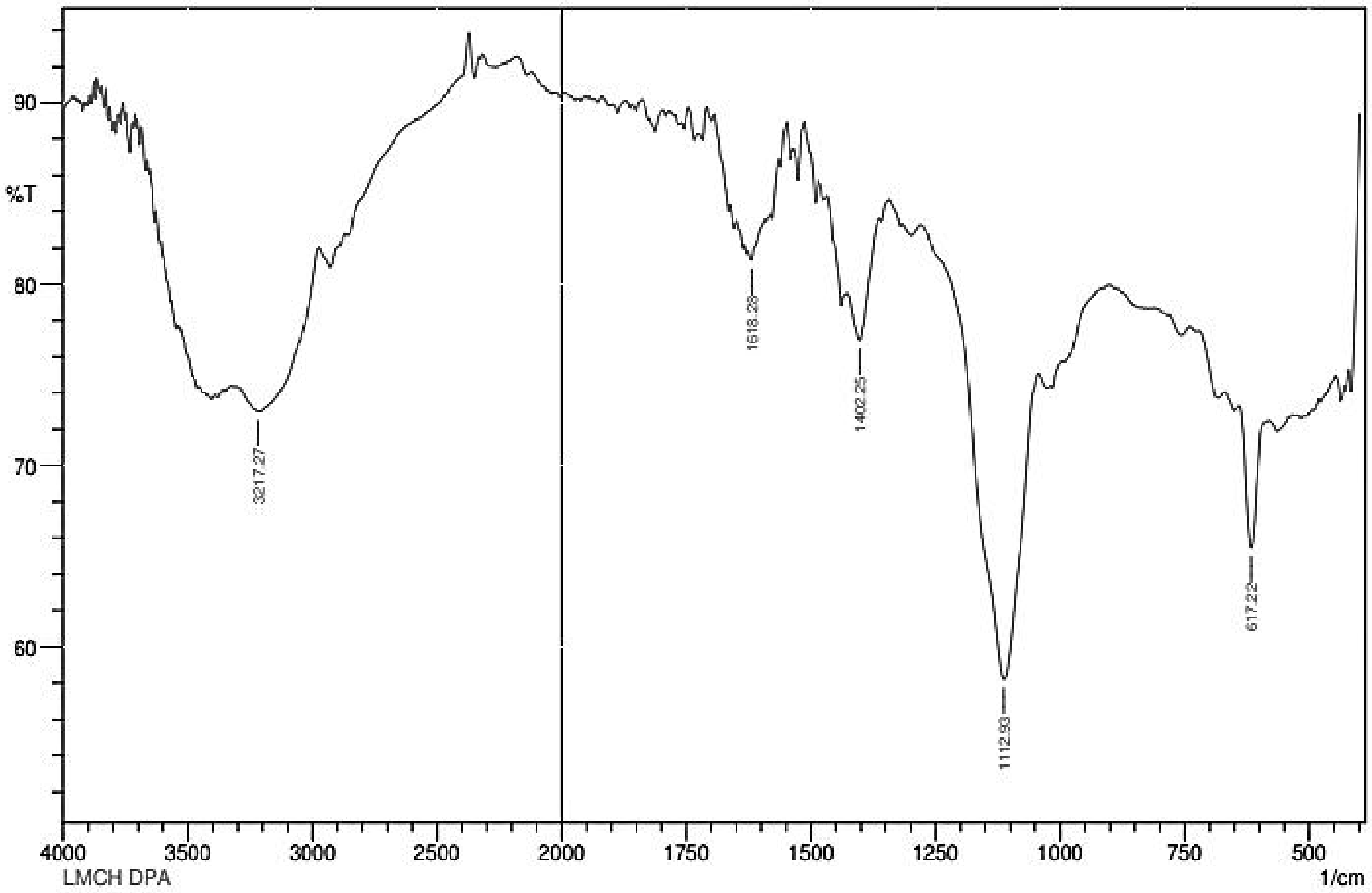


FIGURE 3: FT-IR of dipicolinic acid grafted chitosan



4.2.2 TGA ANALYSIS

TG analysis of MA-g-CH, PCA-g-CH and DPA-g-CH are shown in **figure 4, 6 and 8** respectively.

Combined DTA, DTG and TGA analysis of MA-g-CH, PCA-g-CH and DPA-g-CH are shown in **figure 5, 7 and 9** respectively.

4.2.2.a Mandalic acid grafted chitosan

TG analysis graph MA-g-CH is illustrated in **figure 4** shows two stages of depression. In the first depression curve, the thermal degradation of polymer shows depression about 120°C which is due to the evaporation (loss of water) in the polymer. The weight lost was 35.8%. The second depression for MA-g-CH was appeared between 280°C to 720°C and lost about 24.3% from the original weight. The observed behavior impute to the degradation of acetylated unit of chitosan or depolymerization.**(M Tamer *et. al.,* 2015)**

Further, DTA, DTG and TGA combined graph of MA-g-CH shown in **figure 5**. MA-g-CH exhibited exothermic peak at 610°C and endothermic peak at 700°C. Based on the obtained data, T_{max} value of MA-g-CH was 220°C. The overall weight lost is about 73.7%. **(Chen Li *et. al.,* 2020)**

4.2.2.b p-coumaric acid grafted chitosan

TG analysis graph PCA-g-CH is illustrated in **figure 6** shows two stages of depression. In the first depression curve, the thermal degradation of polymer shows depression about 150°C which is due to the evaporation (loss of water) in the polymer. The weight lost was 26.4%. The second depression for PCA-g-CH was appeared between 280°C to 710°C and lost about 26.9% from the original weight. The observed behavior impute to the degradation of acetylated unit of chitosan or depolymerization. .**(M Tamer *et. al.,* 2015)**

Further, DTA, DTG and TGA combined graph of PCA-g-CH shown in **figure 7.** PCA-g-CH exhibited exothermic peak at 590°C and endothermic peak at 690°C. Based on the obtained data, T_{max} value of PCA-g-CH was 250°C.The overall weight lost is about 70.6%. **(Chen Li *et. al.,* 2020)**

4.2.2.c Dipicolinic acid grafted chitosan

TG analysis graph DPA-g-CH is illustrated in **figure 8** shows two stages of depression. In the first depression curve, the thermal degradation of polymer shows depression about 150°C which is due to the evaporation (loss of water) in the polymer. The weight lost was 31.2%. The second depression for DPA-g-CH was appeared between 280°C to 690°C and lost about 25.1% from the original weight. The observed behavior impute to the degradation of acetylated unit of chitosan or depolymerization. .**(M Tamer *et. al.,* 2015)**

Further, DTA, DTG and TGA combined graph of DPA-g-CH shown in **figure 9.** DPA-g-CH exhibited exothermic peak at 580°C. Based on the obtained data, T_{max} value of MA-g-CH was 300°C. The overall weight lost is about 73.1%.**(Chen Li *et. al.,* 2020)**

FIGURE 4: TG of mandalic acid grafted chitosan

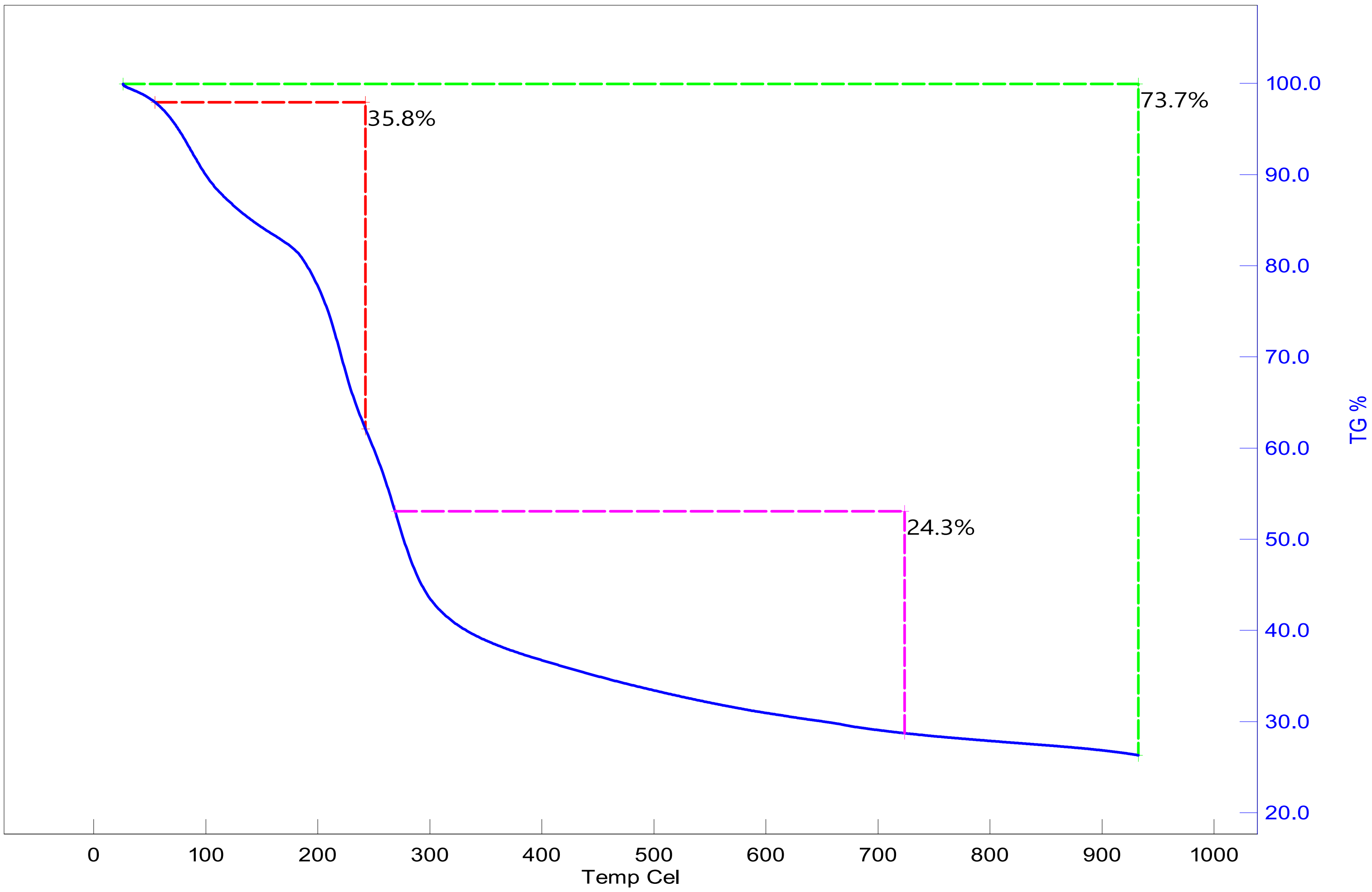


FIGURE 5: DTA,DTG and TGA of mandalic acid grafted chitosan

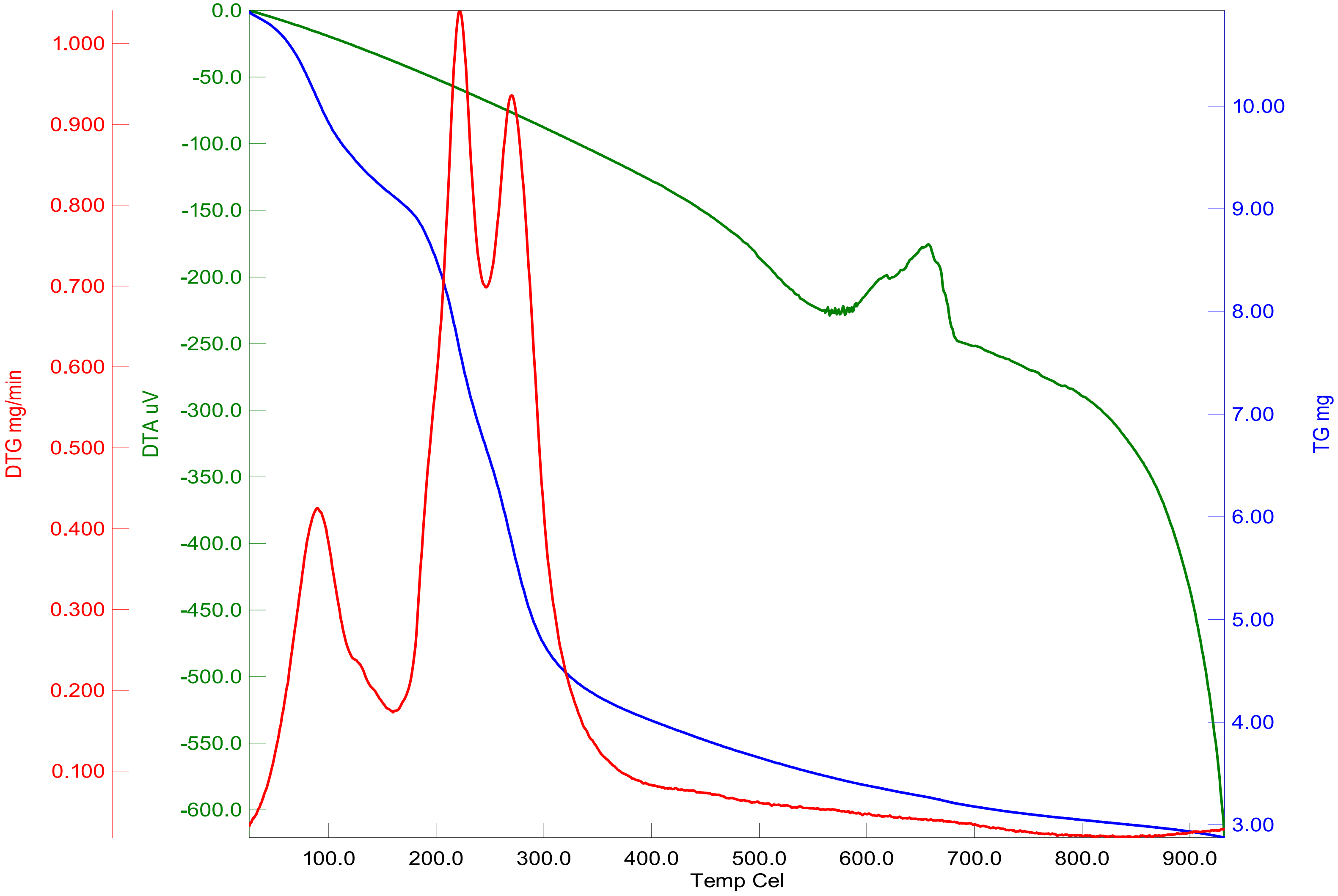


FIGURE 6: TG OF p-coumaric acid grafted chitosan

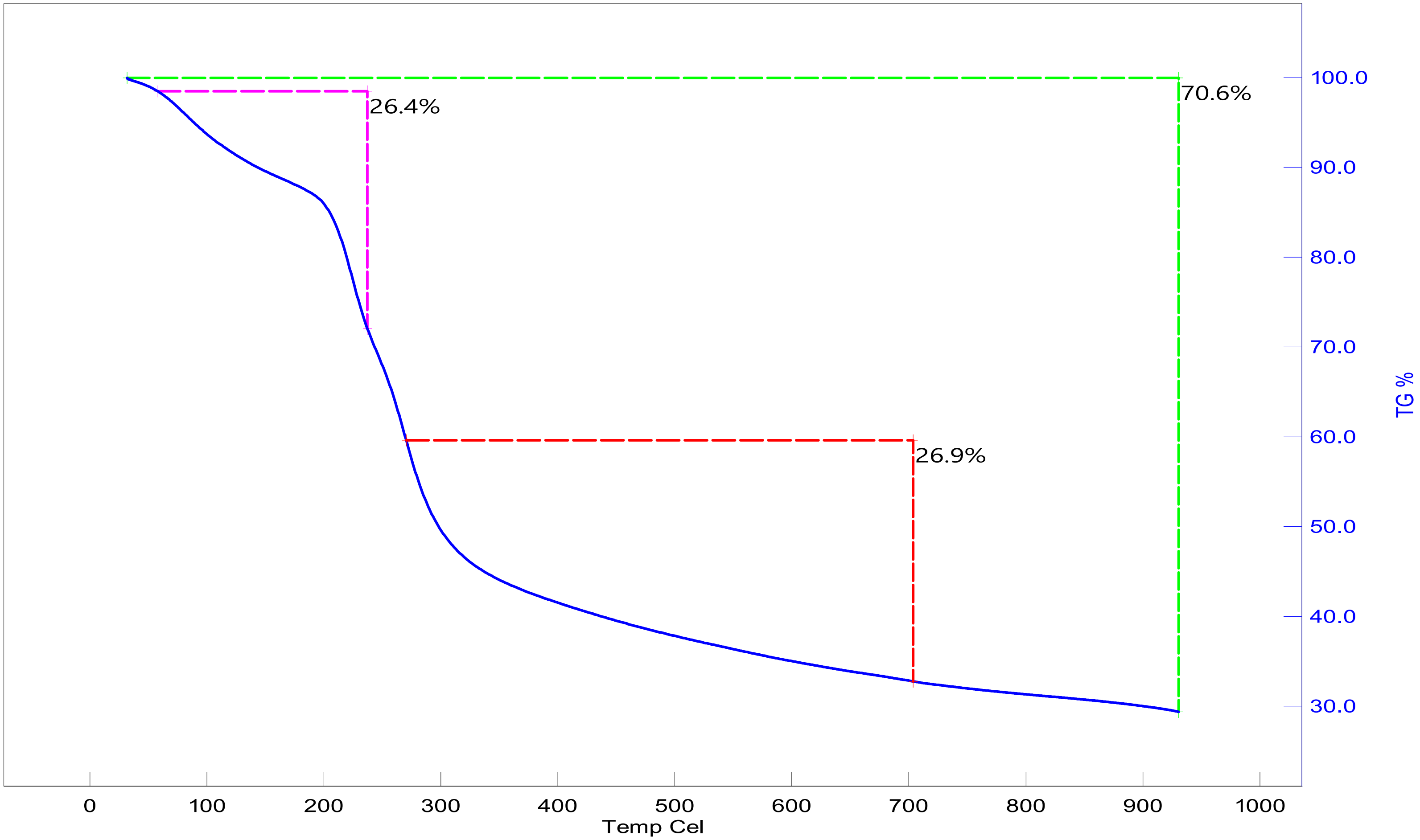


FIGURE 7: DTA,DTG and TGA of p-coumaric acid grafted chitosan

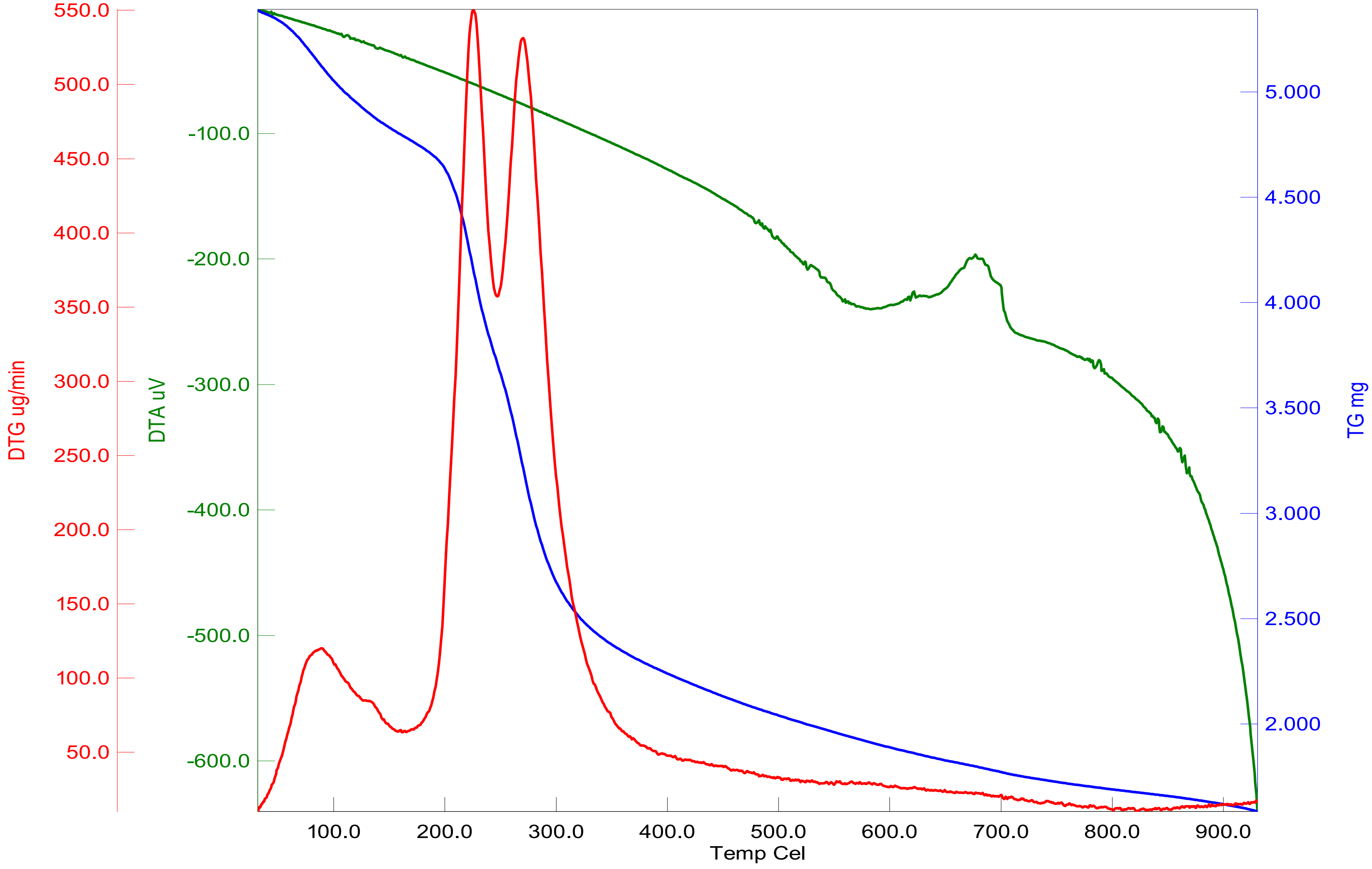


FIGURE 8: TG of dipicolinic acid grafted chitosan

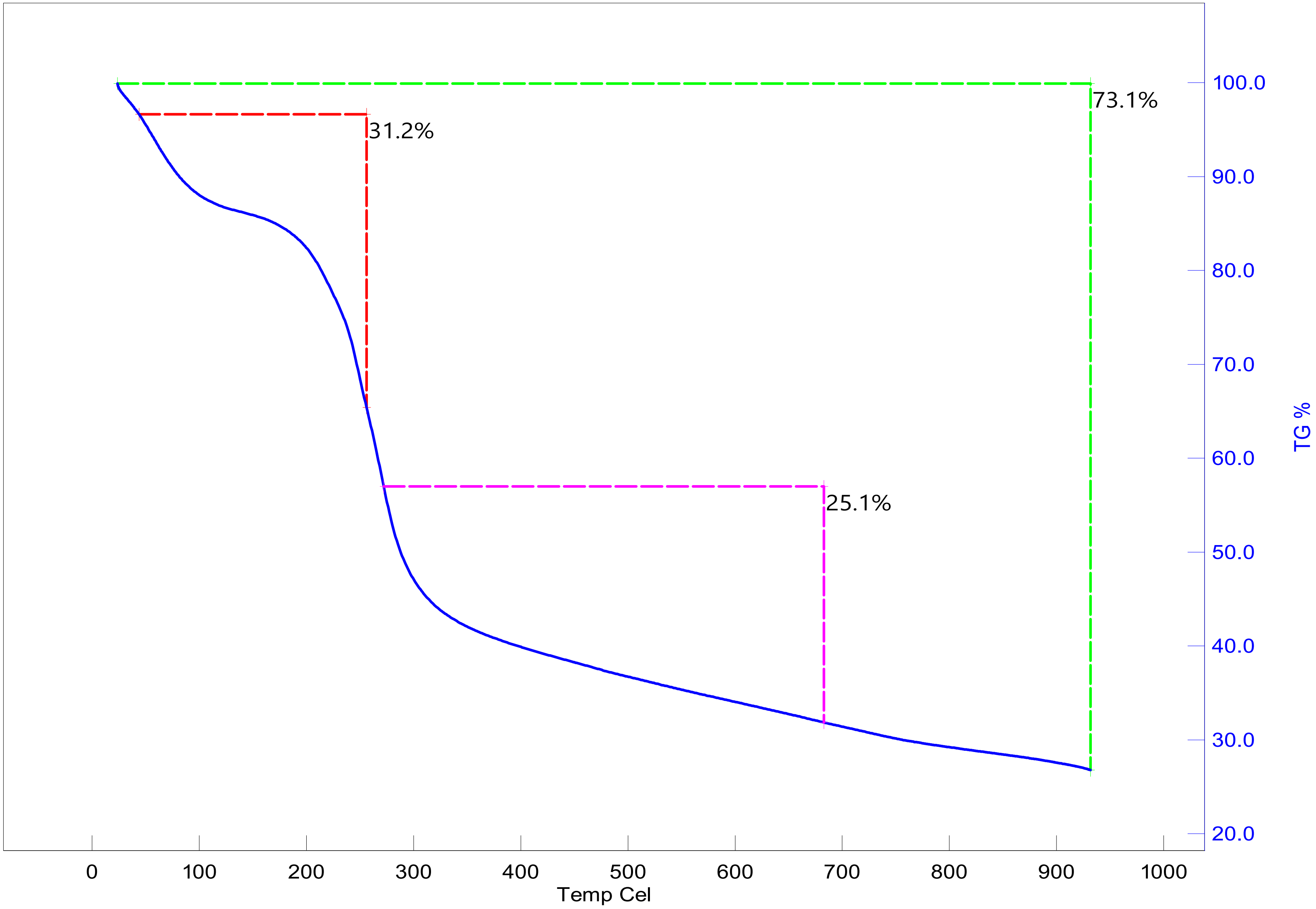
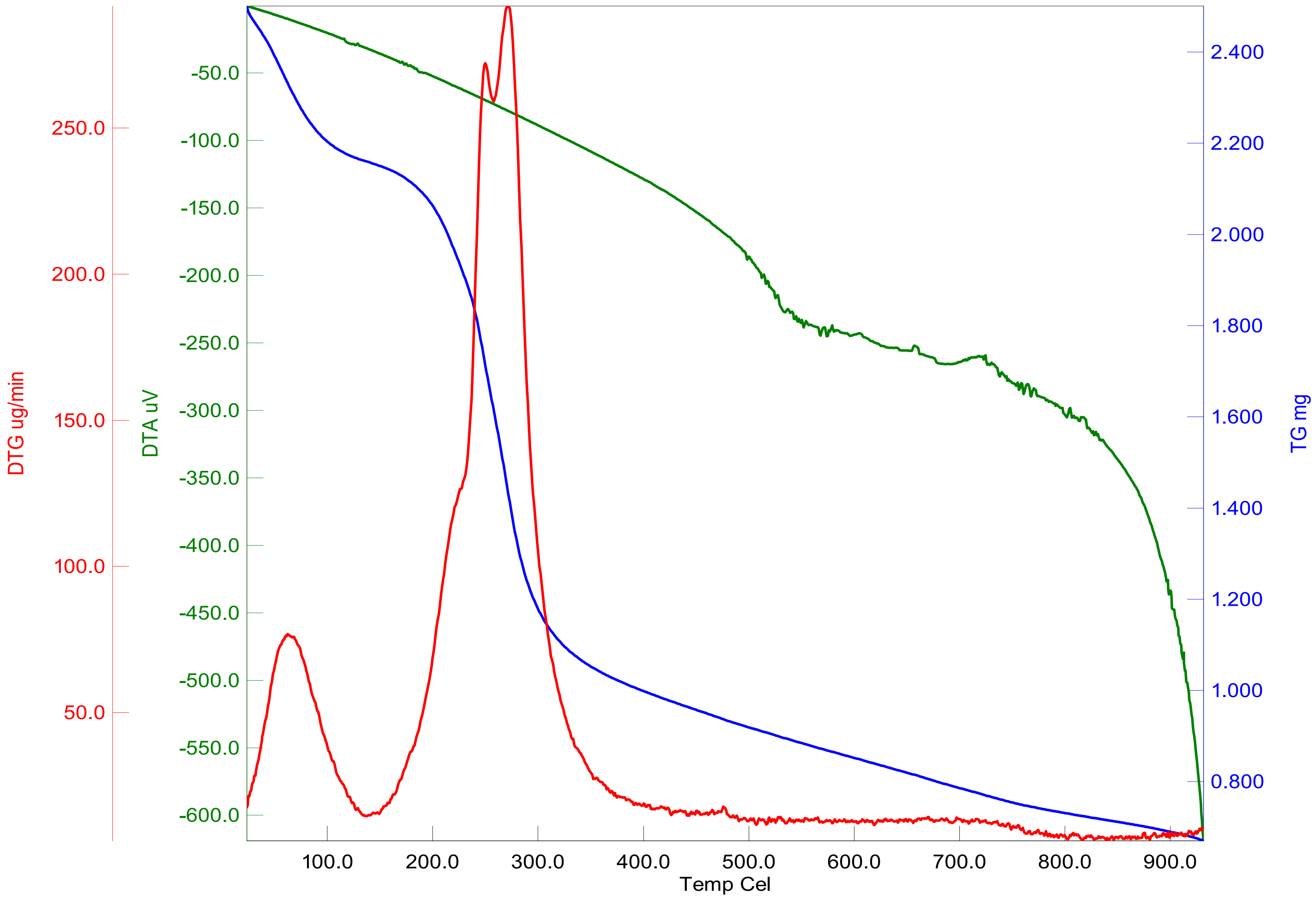


FIGURE 9: DTA,DTG and TGA of dipicolinic acid grafted chitosan



4.2.3 SOLUBILITY

Chitosan, dissolves in acetic acid, does not dissolve in water due to the presence of anime group in their structure. Basically, amine is a base, chitosan dissolve in any solution with low pH. The solubility of chitosan is increased by grafting with acids. The increseed in solubility is due to the formation of amide linkage. The obtained acid grafted polymers showed solubility in water with heating by water bath for a certain minutes.

4.2.4 SURFACE ANALYSIS

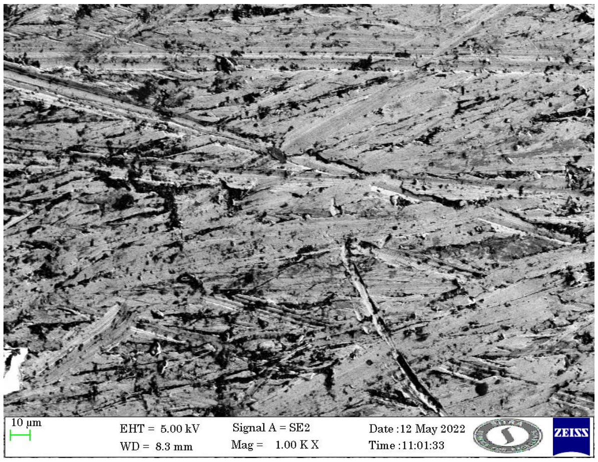
- The surface features of 316L stainless steel sample without coating and with coating was analyzed in ZEISS SEM analyzer, the micrographs are shown in **figure 10-16**.

- The SEM image of plain stainless steel (blank) shown in **figure10**. The image showed no cracks and coating, only the surface of the steel was observed.

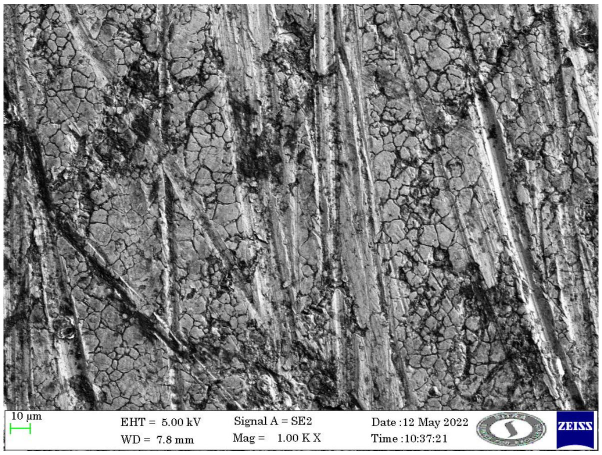
- The acid grafted chitosan polymer (pH 2) is coated 316L stainless steel images are shown in **figure 11,12 and 13.** The surface showed gray pits which implies, acid grafted chitosan polymer has been coated over 316L stainless steel surface.

- The SEM images of 316L stainless steel coated at pH 4 shown in **figures 14,15 and 16**. It shows visible fissures when compared to SEM images of 316L stainless steel taken at pH 2. This indicated the coating of polymer was not uniform at higher pH.

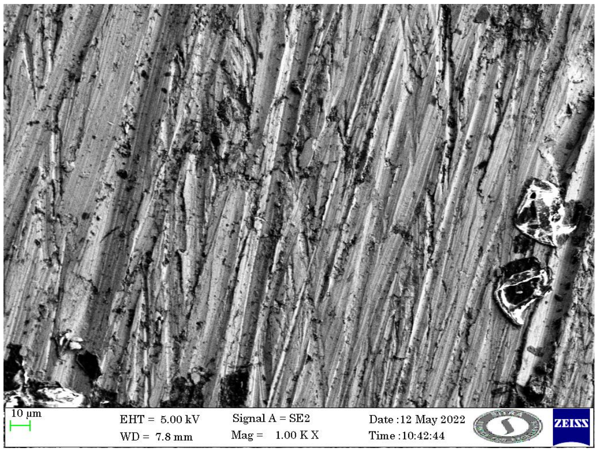
SEM micro graphs of Blank and Acid grafted Chitosan coated 316L Stainless Steel



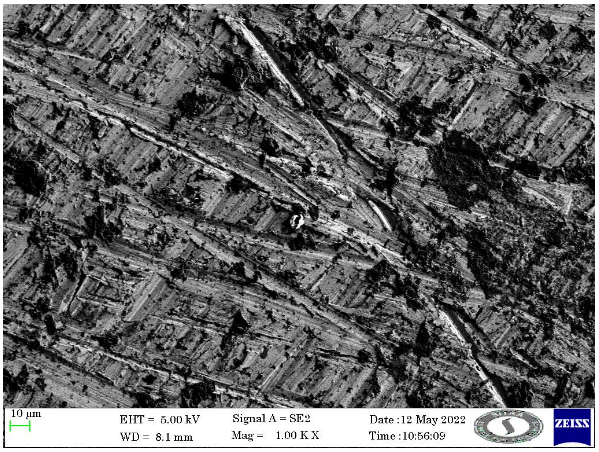
(10)



(11)



(12)



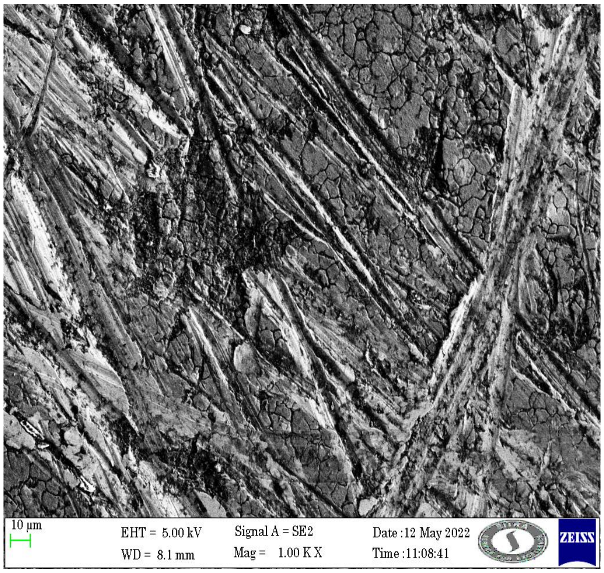
(13)

FIGURE 10: SEM image of 316L stainless steel without coating

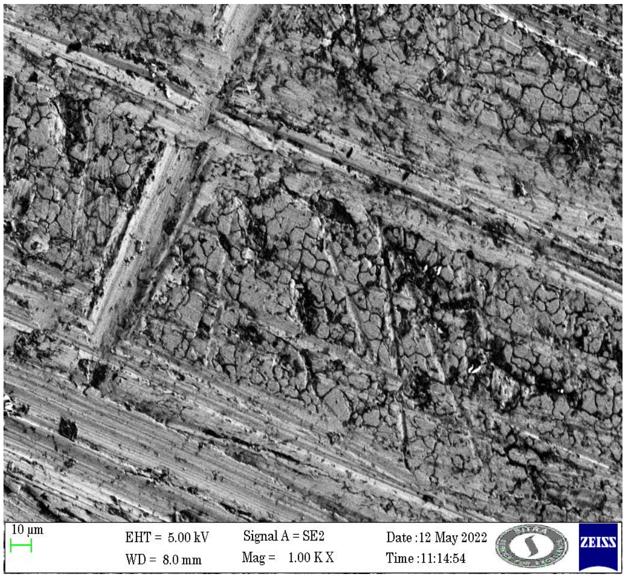
FIGURE 11 : SEM image of 316L stainless steel with MA-g-CH coating

FIGURE 12: SEM image of 316L stainless steel with MA-g-CH coating with the pH of 4

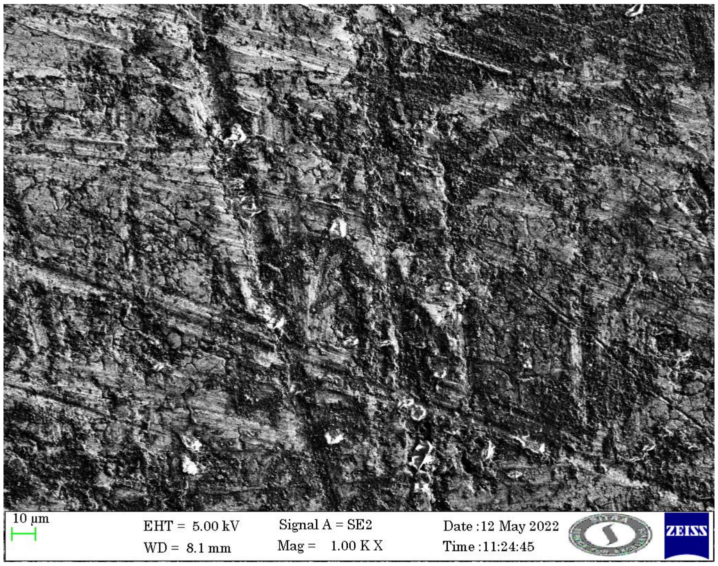
FIGURE 13: SEM image of 316L stainless steel with PCA-g-CH coating



(14)



(15)



(16)

FIGURE 14: SEM image of 316L stainless steel with PCA-g-CH coating with the pH of 4

FIGURE 15: SEM image of 316L stainless steel with DPA-g-CH coating

FIGURE 16: SEM image of 316L stainless steel with DPA-g-CH coating with the pH of 4

CONCLUSION
AND
SUMMARY

5. SUMMARY AND CONCLUSION

The results of the coating of acid grafted chitosan polymeric film in different condition are summarized below,

- The preparation of acid grafted chitosan is successful in presence of ammonium per sulphate.
- The structure of the compound is confirmed by FT-IR and TGA
- The solubility of the acid grafted chitosan is increased compared with chitosan in water.
- The sol gels of the respective acid grafted polymers are successfully coated on 316L stainless steel at two different pH 2 and 4.
- SEM analysis is carried out for all the acid grafted chitosan coated 316L stainless steel to confirm the coating performed at pH 2 is better than coating done at pH 4.
- Further, the films can be analyzed for antimicrobial, antibacterial studies so that this can be used in implants.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