Modification of natural polysaccharides via grafting and cross-linking and their applications

THESIS

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DEDICATED TO

MY FATHER AND MOTHER

CERTIFICATE

This is to certify that the thesis entitled "**Modification of natural polysaccharides via grafting and cross-linking and their applications**" submitted by Ms/Mr. **Deepak Kumar** is an original research work and has not been previously submitted in part or full for the award of any other degree or diploma to this or any other university.

The thesis submitted to Babasaheb Bhimrao Ambedkar University Lucknow satisfies all the requirements as stipulated in the Doctor of Philosophy (Ph.D.) regulation-1999 as amended in 2008/2010/2013 and it is fit for submission and evaluation for the award the of the degree of Doctor of Philosophy of the University.

23/08/19

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DECLARATION

I hereby declare that the thesis entitled "**Modification of natural polysaccharides via grafting and cross-linking and their applications**" submitted for the degree of Doctor of Philosophy (Ph.D), is the record of work carried out by me under the supervision of **Dr, Jyoti Pandey, Assistant professor, Department of Applied Chemistry, School For Physical Sciences,** Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, India and co-supervision of **Dr. Pramendra Kumar, Assistant Professor, Department of Applied Chemistry, M.J.P. Rohilkhand University, Bareilly, India** and I further confirm that the work has not been published anywhere else for the award of any degree, diploma, fellowship etc. either in this or any other University or other institution of higher learning. I further declare that the material obtained from other sources has been duly acknowledged in the thesis. I also undertake that the thesis is essentially free from all kinds of plagiarism.

24/8/18

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***

ABSTRACT

Polysaccharides are carbohydrate polymers in which monosaccharide [$(CH_2O)_n$] units are covalently joined by *O*-glycosidic bond in either a branched or linear configuration. Polysaccharides serve as stores of energy, as in glycogen (branched polysaccharide of glucose), and as a structural component of bacterial cell walls, as cellulose (linear polysaccharide of glucose). Natural polysaccharides have the properties of artificial counterparts and in addition being essentially biodegradable, renewable, non-toxic, and comparatively cheap. Their characteristics at the structural level are associated with their hydrogen-bonding ability, side-group reactivity with either primary or secondary linkages.

Polysaccharides have been extensively used in various fields owing to their remarkable chemical, biological and physical properties. Herein, the applications of polysaccharides are introduced, specifically in wound healing, targeted delivery, bio-sensing, conducting polymer, catalysis and agents with antimicrobial, antiviral, anticancer capabilities, in water treatment and many industries such as textile, paper, rubber, plastic and pharmaceutical industries etc. In spite of the significant chemical and physical properties of polysaccharide, biodegradability and short shelf life, are posing problems in the commercialization of the utility of polysaccharide. The grafting of synthetic monomer/polymers onto natural polysaccharides is a popular, significant and convenient route to modify the polysaccharide. There are two main grafting methods: one is the conventional method and another is microwave irradiation method. In conventional grafting process, an inert atmosphere is required along in the long reaction time whereas there is no need of inert atmosphere in microwave irradiation process and it can happen in seconds. Indeed, increasing interest in clean and green environmentally friendly chemistry has motivated the use of microwaves in the polysaccharide grafting modification for various applications. Microwave irradiation significantly reduces the use of toxic solvents, as well as the reaction time for almost all the grafting reactions of interest here, ensuring high yields, product selectivity and clean product formations.

The whole research work is divided into five chapters. The first chapter comprises the general introduction of polysaccharides and graft/crosslink copolymer preparation methods including Microwave irradiation and conventional methods. The exhaustive literature review on the topic and the objective of the present work has been discussed. In the chapter the general characteristics and applications of graft/crosslink copolymer and the various

instrumentation techniques are discussed for identification of prepared graft/crosslink materials.

In the second chapter, a binary grafted copolymer of Psyllium mucilage (Psy) with acrylic acid (AA) and acrylonitrile (An) has been successfully synthesized under microwave conditions for *in vitro* drug release study. The grafting was confirmed by FTIR spectroscopy, XRD, SEM, EDX, TGA analytical techniques and the intrinsic viscosity studies. The swelling behavior of grafted material has been studied in solutions of different pH and time. We have also prepared Psy-g-Poly (AA-co-An) based beads with the anti-cancer drug [(2-Chloro-3-(4-hydroxyphenylamino) naphthalene-1, 4-dione)]. The drug release behavior of Psy-g-Poly (AA-co-An) based beads has been determined in aqueous medium at different pH, where highest drug release was observed at pH 1.6. The drug release kinetics was analyzed using different models. This study demonstrates that the release of drug depends on the composition of beads and pH of release medium. Kinetics of drug release from beads is best fitted by zero order and first order model.

In the third chapter, we report herein the synthesis of novel antibacterial graft [Chit-g-Poly (AA-co-An)] and crosslink [Chit-cl-Poly (AA-co-An)] copolymer, consisting of acrylic acid (AA), acrylonitrile (An) and chitosan by using the microwave route, where it has been found that grafting and crosslinking copolymers have excellent antimicrobial properties. Studies of antibacterial activities of graft and crosslink samples were carried out against gram-positive [*Staphylococcus aureus (S. aureus*)], gram-negative [*Escherichia coli (E. coli)* and *Pseudomonas aeroginosa (P. aeroginosa*)] bacteria. The graft [Chit-g-Poly (AA-co-An)] and crosslink [Chit-cl-Poly (AA-co-An)] copolymers were characterized by Fourier transform infrared spectroscopy (FTIR), scanning electron micrography (SEM), thermogravimetric analysis (TGA), X-ray diffraction (XRD) techniques to study structural characteristics of synthesized chitosan derivatives. The graft [Chit-g-Poly (AA-co-A)] copolymer shows excellent antibacterial activities against *E. coli*, *P. aeroginosa* and *S. aureus* 30, 31 and 26 mm zone inhibition, respectively meanwhile [Chit-cl-Poly (AA-co-A)] shows antibacterial activities against *E. coli*, *P. aeroginosa* and *S-aureus* 26, 36 and 21 mm zone inhibition respectively.

In the fourth chapter, the antimicrobial binary grafted chitosan film [chit-g-Poly (An-co-Am)] was prepared by grafting of acrylonitrile and acrylamide on to chitosan via microwave initiated graft copolymerization. The grafting of acrylonitrile and acrylamide onto chitosan backbone was confirmed by FTIR, XRD, SEM and TGA/DTA/DTG analytical techniques. The binary grafted chitosan film possessed efficient antimicrobial activity against

three tested strains, i.e. *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The prepared binary grafted chitosan film was tested for packaging of apple and guava to prevent microbial infection and extend their shelf life. The biodegradability study of binary grafted chitosan film was also done and all the results were positive.

In the fifth chapter, Psyllium-g-Poly-(acrylamide-co-acrylonitrile) has been synthesized from psyllium under the N_2 atmosphere, in presence of ceric ammonium nitrate and ascorbic acid couple (CAN/AA) as initiator for adsorption of mercuric ions from synthetic solution of $HgCl_2$. The synthesized samples were optimized by varying synthetic parameters *viz.* monomer concentration, reaction time, temperature, initiator concentration etc. to obtain the maximum yield of grafted product as well as maximum adsorption of ionic mercury. The optimized sample has been characterized through FTIR spectroscopy, SEM analysis, X-Ray diffraction and thermal studies (TGA/DTA/DTG). The mercury adsorption was studied onto the optimized sample and found maximum at temperature (30°C), dose (30 mg), pH (6), time (60 min) and initial concentration of mercury with 100 ppm. Equilibrium isotherm data were analyzed through Langmuir and Freundlich isotherms. Langmuir model was more fitted (R^2=0.9976) which indicated the monolayer sorption. The kinetics of sorption of mercury (II) were also analysed using the first order (R^2= 0.9971), second order (R^2= 0.9887), pseudo-first order (R^2= 0.9971), pseudo-second-order (R^2= 0.9481), intra-particle diffusion (R^2= 0.9958) and Elovich equation (R^2=0.9624). Second order rate kinetics has best linearly fitting, which follows the chemisorption mechanism.

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List of Abbreviation

AA	Acrylic acid
An	Acrylonitrile
Am	Acrylamide
-g-	Grafting
-cl-	Crosslinking
CAN	Ceric ammonia nitrate
FAS	Ferrous ammonium sulfate
KPS	Potassium persulfate
PAM	Polyacrylamide
MW	Microwave
Psy	Psyllium
Chit	Chitosan
GG	Guar gum
MBC	Minimum concentration of bactericidal
SEM	Scanning Electron Microscopy
TGA	Thermal gravimetric analysis
DSC	Differential scanning calorimetric
MeOH	Methyl alcohol
MeCOMe	Acetone
FTIR	Fourier-transform infrared spectroscopy
Ppm	Parts per million
M	Monomer
MO	Methyl Orange
MB	Methylene Blue
NPs	Nanoparticles
E. coli	Escherichia coli
P. aeroginosa	Pseudomonas aeroginosa
S. aureus	Staphylococcus aureus

Chapter -1

Introduction and Literature review

Polysaccharides have been extensively used in various fields owing to their remarkable chemical, biological and physical properties. Herein, the applications of polysaccharides are introduced, specifically in wound healing, targeted delivery, bio sensing, conducting polymer, catalysis and agents with antimicrobial, antiviral, anticancer capabilities, in water treatment and many industries such as textile, paper, rubber, plastic and pharmaceutical industries etc. In spite of the significant chemical and physical properties of polysaccharide, biodegradability and short life, are posing problems in the commercialization of the utility of polysaccharide. The grafting/cross-linking of synthetic polymers onto natural polysaccharides is a popular, significant and convenient route to modify the polysaccharide. The general introduction of polysaccharides, properties, applications, and the various grafting methods including microwave irradiation and conventional methods of modification of natural polysaccharides have been discussed in this chapter. The exhaustive literature review on the topic and the objective of the present work has been discussed. This chapter also containing the general characteristics and applications of graft/crosslink copolymer and the various instrumentation techniques are discussed for identification of prepared graft/cross-link materials.

1.1. Introduction

Natural polymer mainly polysaccharides are abundant in nature [1] and readily obtained from almost every living organisms [2] such as trees, seashells, microbial resources, animal body fluids [3], shells of crustaceans [4], insects [5] and are also present in cell walls as well as cellular fluids of fungi, yeast and bacteria [4]. Polysaccharides are inexpensive natural polymers of monosaccharides. Polysaccharides are found in various structures, therefore, they demonstrate the various physical and chemical properties [6]. Various useful and beneficial polysaccharides are prepared by biotechnical routes [7].The polysaccharide can be modified easily and are used as a targeted drug delivery system because it is highly stable, safe, non-toxic, hydrophilic, biodegradable and have excellent gel-forming properties [8].
Polysaccharides are carbohydrate polymers in which monosaccharide [$(CH_2O)_n$] units are covalently joined by *O*-glycosidic bond in either a branched or linear configuration. Polysaccharides may store energy in the form of glycogen (branched polysaccharide of glucose), or act as a structural component of bacterial cell walls in cellulosic form (linear polysaccharide of glucose) [9]. Their characteristics at the structural level are associated with their hydrogen-bonding ability, side-group reactivity with either primary or secondary linkages [10].

Polysaccharides that are omnipresent can either be homo or hetero polysaccharides and obtained from algae, plants [11], microbes and animals [12]. Hydrophilic groups (hydroxyl, carboxyl and amino groups) in polysaccharide form non-covalent bonds with biological tissues (epithelia and mucous membranes) forming bio-adhesion [13]. Polysaccharides are divided into three main categories one is positive charged polysaccharides for e.g. chitosan, second is negative charged polysaccharides for e.g. psyllium, heparin, alginate, pectin and hyaluronic acid and third is neutral polysaccharides for e.g. starch, dextrins, guar gum [14]. It has been found that polysaccharides, along with oligosaccharides is an abundant set of biopolymers that contribute in various important biological processes, like cell-cell communication, embryonic expansion, infection of bacteria or viruses and cellular immunity [15].

The natural polymer holds an advantage over the artificial polymers due to biodegradability, availability, easy storage, non-toxic nature and cost-effectiveness but the major disadvantage is their short shelf life, which can be improved via chemical modifications [7]. The presence of several derivable functional groups in the molecular

skeleton of polysaccharides enables their modifications by biochemical and chemical means to introduce the desirable properties [16] which enhance the stability and utility of polysaccharides. Polysaccharides derived biodegradable polymers exhibit wide applications in various fields like biomedical, pharmaceutical and environmental fields [15]. The grafting and crosslinking are the two important methods which address the drawbacks of polysaccharides i.e. short shelf life and great water solubility by adjusting the solubility while maintaining biodegradability [17]. These modified polysaccharides are widely used as a coating agent, gelling agent, antimicrobial agent [2], stabilizer, biomedical materials, biomimetic optical nanomaterials, polymer electrolytes [18], absorbent as well as adsorbent [19] and also used in paper, cosmetics, textiles [9], food [5], biomedical [20] and pharmaceutical industries [9] as drug delivery candidate. Polysaccharides such as chitosan, cyclodextrins, chondroitin sulfate, dextrin, guar gum, pectin, locust bean gum and amylose [21] are applicable to controlled drug delivery, mostly for prolonged [7] time-release of drug and also increase the activities of labile medicines [22]. Therefore many researchers took the interest to modify the natural polysaccharide to obtain their grafted/crosslinked derivatives through the grafting/crosslinking of the small monomer on to the polysaccharide backbone because these modified polysaccharides exhibit various applications in different fields. Grafting and crosslinking are the easiest ways to make the compatibility [7] between artificial polymers and original polysaccharide [23].

1.2. Polysaccharides

1.2.1. Psyllium mucilage

Psyllium, a natural ayurvedic herb cultivated in Asian countries, have profound application in health sector because of its medicinal properties [24]. India is the highest producer [25, 26] and exporter of psyllium world-wide. Psyllium is also known as Ispaghula and isabgol. Isabgol is made from Persian words band ghoul, meaning [27] “horse flower" and descriptive the shape of the seed. Psyllium is a natural carbohydrate obtained from Plantago plant’s seed husk through mechanical milling or grinding process and used after purification. Psyllium mucilage is produced from various classes of plantaginaceae family including *Plantago asiatica L.* [28], *P. ovata* [29], *Plantago depressa*, *Plantago lanceolata*, and *Plantago palmata, P.* [30]. *P. ovata* is a rich source of psyllium mucilage [25, 28], both the seeds and leaves of the *P. ovata* have been used to enclosed medicine for a long time [28]. It is

extensively used as a household medicine since ancient time in all cultures, against various types of diseases [31].

Psyllium polysaccharide (Fig.1.) is an anionic polysaccharide which consist of arabinoxylan units [7, 32] (arabinose 22.6% [25, 33], xylose 74.6%) [34]. Psyllium is highly mucilaginous food grade fibre because it has the greatest capability to form a gel with water and the gel-forming fraction, amounting of psyllium husk approximate 55–60% which is responsible for the laxative and cholesterol-lowering [35]. Some polysaccharide based studies confirmed that enhanced fibre consumption may reduce the possibility of colon cancer. Psyllium mucilage is also bulking fibre, after absorption, it expands and it forms a gel with water in the colon [36].

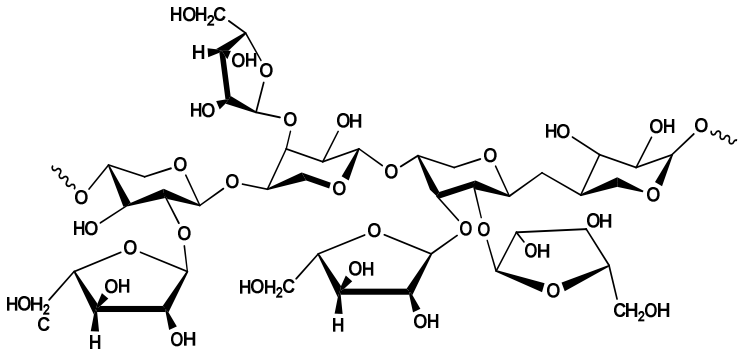

Fig.1. Structure of psyllium.

Psyllium mucilage is very valuable for medicinal [37] and pharmaceutical applications. It is generally treated like a laxative, which has a plethora of health benefits and has proven to be effective in treating irritable, constipation, diabetes, bowel syndrome, high cholesterol, colon cancer, obesity, atherosclerosis and ulcerative colitis among several other health conditions.

1.2.2. Alginates

Alginate is a significant member of polysaccharides family [38, 39] because of high effectiveness towards the hydrogel formation at various pH and temperature and also exhibit excellent biocompatibility and biodegradability, having different applications in biomedical field [40, 41]. Alginate is readily processable for applicable three-dimensional scaffolding materials [11] such as hydrogels, microspheres, microcapsules, sponges, foams and fibers [42]. Alginate-based biomaterials can be utilized as drug delivery systems and cell carriers for tissue engineering [43].

Alginates are a group of naturally occurring anionic polysaccharides derived from brown algae cell walls, including *Macrocystis pyrifera*, *Laminaria hyperborea*, *Ascophyllum nodosum* [44] and several bacteria strains (*Azotobacter*, *Pseudomonas*) [42]. This term usually referred to alginic acid and its salts [45], but it can also be used for all derivatives of alginic acid [46]. Alginates are linear biopolymers consisting of (1→4) linked β-D-mannuronic acid (M) and (1→4) α-L-guluronic acid (G) residues (Fig. 2) arranged in homogenous (poly-G, poly-M) or heterogenous (MG) block-like patterns [45, 47]. With regard to the initial source material, commercial alginates may differ in composition and the sequence of G and M blocks [48]. Natural polymer composition, sequence and molecular weight vary greatly depending on the source and species producing this natural polymer [43, 49]. Therefore, there is an important additional possibility to design a maintainable bio-compound based on natural alginates. The combination biochemical as well as chemical techniques offers considerable probability for generating improved alginic acid compounds with control [43] over monomer units sequence and nature, location and extent of substituents.

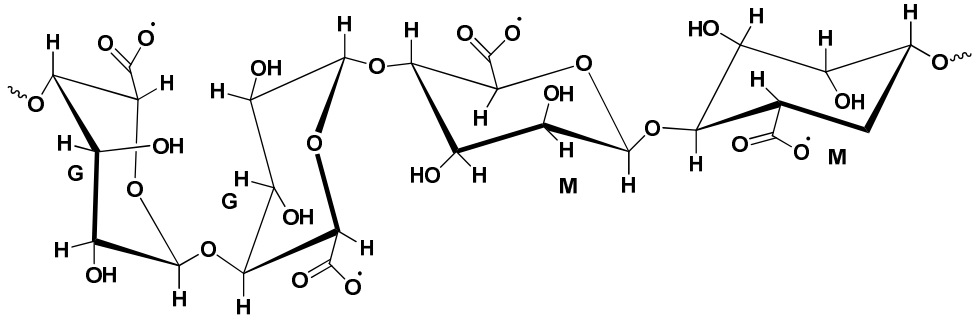

Fig.2. Structure of alginate.

Alginate is of particular interest for a broad range of applications as a biomaterial and especially as the supporting matrix or delivery system for tissue repair and regeneration. Due to its outstanding properties in terms of biocompatibility, biodegradability, non-antigenicity and chelating ability, alginate has been widely used in a variety of biomedical applications including tissue engineering, drug delivery and in some formulations preventing gastric reflux [50]. To chelate with divalent cations is the easiest way to prepare alginate hydrogels from an aqueous solution under gentle conditions [43, 51]. As a result of the naturally occurring polysaccharide, alginate exhibits a pH-dependent anionic nature and has the ability to interact with cationic polyelectrolytes and proteoglycans. Therefore, delivery systems for

cationic drugs and molecules can be obtained through simple electrostatic interactions [52]. More significantly, the utility of alginate crosslinking to create hydrogels for cell encapsulation has demonstrated to be most beneficial for biomedical applications [53-55].

1.2.3. Chitin

Chitin, a naturally abundant mucopolysaccharide and the supporting material of crustaceans, insects, etc., is well known to consist of 2-acetamido-2-deoxy-β-D-glucose through a β (1→4) linkage. Chitin can be degraded by chitinase [56]. Chitin is a highly insoluble material resembling cellulose its solubility and low chemical reactivity [57]. It is considered as a cellulose derivative [58]. Chitin has a similar structure (Fig. 3) as cellulose, but at the C_2 position, it has one acetamide group (-$NHCOCH_3$). Chitin is a natural, bio-renewable, eco-friendly, biodegradable, bio-functional polysaccharide and beneficial chelating agent. It is widely used as water treatment additive, drug carrier and other important applications [59]. However, now days, chitin is not vastly employed by the pharmaceutical industry because of its less solubility in water. Chitin is almost insoluble in common organic solvents and diluted aqueous solvents because it is highly hydrophobic due to its highly expanded hydrogen-bonded semi-crystalline structure. Chitosan is the main derivative of chitin and obtained by deacetylation of chitin.

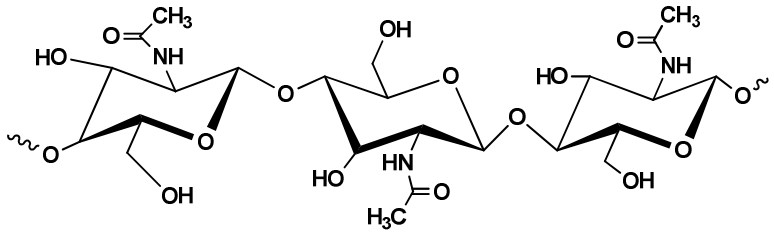

Fig.3. Structure of chitin.

1.2.4. Chitosan

Chitosan is one of the most prominent candidates among biopolymers, which has attracted more attention mostly due to its antibacterial, antifungal properties and also has the high metal-binding capacity. Chitosan is a cationic polysaccharide obtained by a full or partial deacetylated of chitin, chitosan consists of (1→4)-linked β-D-glucosamine $(C_6H_{11}O_4N)_n$ [60] (N-acetyl glucosamine) [61] units (Fig. 4) [62-64]. Chitosan is white to light red solid powder, insoluble in water but soluble in organic acids. Due to its non-toxic, biocompatible, mucoadhesive and biodegradable, antibacterial, antifungal and metal-binding

properties [65, 66]. In 1983 and 1995 Korea and Japan respectively, have established the chitosan as a food additive [67-69].

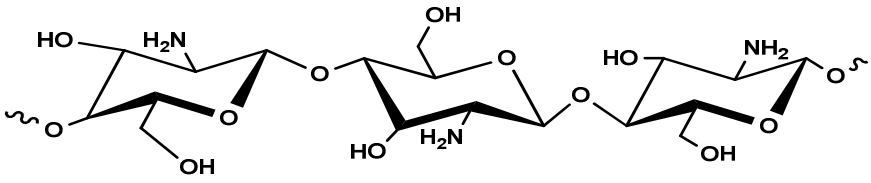

Fig.4. Structure of chitosan.

Chitosan, is a biological active natural polysaccharide obtained, possesses various biological properties, such as cytocompatibility [70], antimicrobial [71], antioxidant, anti-cholesterolemic [72], anti-inflammatory, analgestic [73], haemostatic, mucoadhesion [74]. Chitosan has been used in a variety of applications, most relevant in the medical as well as pharmaceutical fields [75].

1.2.5. Guar gum

Guar gum polysaccharide is obtained from the seed of the tolerant plant *Cyamopsis tetragonoloba* [76], belongs to the *Leguminosae* family [66]. The common names used in the scientific literature for the bean, guar gum flour and the galactomannan fraction are Indian cluster bean, guar and guarana, respectively [66]. The guar gum is composed of several layers, namely the outer husk (16–18%), the germ (43–46%) and the endosperm (34–40%). The germ portion of its seed is predominantly protein and the endosperm predominantly galactomannan [66]. Guar gum mainly consists of the high molecular weight polysaccharides of galactomannans which are linear chain of (1→4)-linked β-D-mannopyranosyl [77] units with (1→6) linked α-D-galactopyranosyl [78] residues as side chains (Fig. 5).

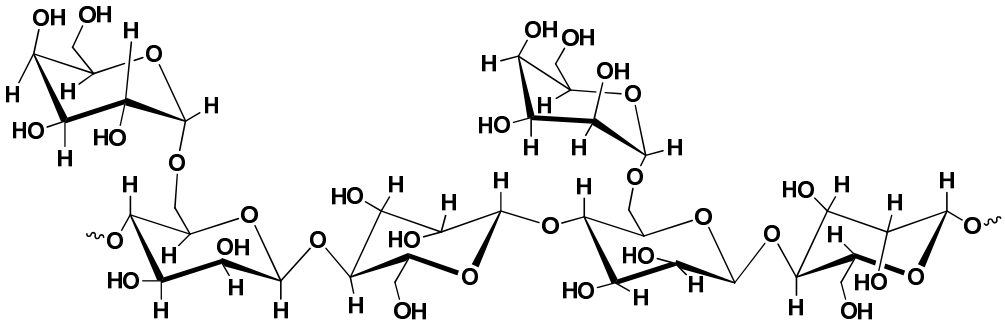

Fig.5. Structure of guar gum.

Natural guar gum is the highest molecular weight polysaccharide out of totally naturally occurring [79] water-soluble polymers. Guar gum has some special physical and chemical properties which make it a powerful candidate in the world of excipients in food, cosmetics and pharmaceuticals. Natural guar gum polysaccharide and its derivatives are highly used in therapy [66]. The chemistry of guar gum permits it to be modified by chemically to obtain derivatives of necessary properties that are inexpensive, eco-friendly and biodegradable. It is a safe and non-hazardous natural polysaccharide; obtain from renewable natural resources [80]. Guar gum is largely used in the form of guar gum powder as an additive in food, pharmaceuticals, paper, textile, explosive, oil well drilling and cosmetics industry [66]. Industrial applications of guar gum are possible because of its ability to form hydrogen bonding with water molecule [80]. Thus, it is chiefly used as a thickener and stabilizer. It is also beneficial in the control of many health problems like diabetes, bowel movements, heart disease and colon cancer [81].

1.2.6. Gum Ghatti

Gum Ghatti is obtained from the *Anogeissus latifolia* tree, also famous as India gum. *Anogeissus latifolia* is a kind of trees of the *combretaceae* family [82]. *Combretaceae* family are found in Africa and the in Arabian Peninsula of South Asia. The commercial importance of gum ghatti became obtainable due to the establishment of plantations in India. Gum ghatti has excellent emulsification properties and the emulsification capacity is greater than gum arabic or other natural polysaccharides [82].

Gum ghatti is an original polysaccharide which is obtained from nature as combined with Ca and Mg salts of uronic acid and ghattic acids [21] and also has 3% protein. It is made up of L-arabinose, D-mannose, D-galactose, D-glucuronic acid and D-xylose in a 48: 10: 10:5: 29 molar ratio and less than 1% of rhamnose [83]. It has (1→6) linked β-D galactopyranose [84] units and 4-*O*-substituted and 2-*O*-substituted α-D-mannopyranose units [85] with side chains of L-arabinose units [85]. Gum ghatti was obtained by a combination of 1900 units of gum Arabic (Fig. 6).

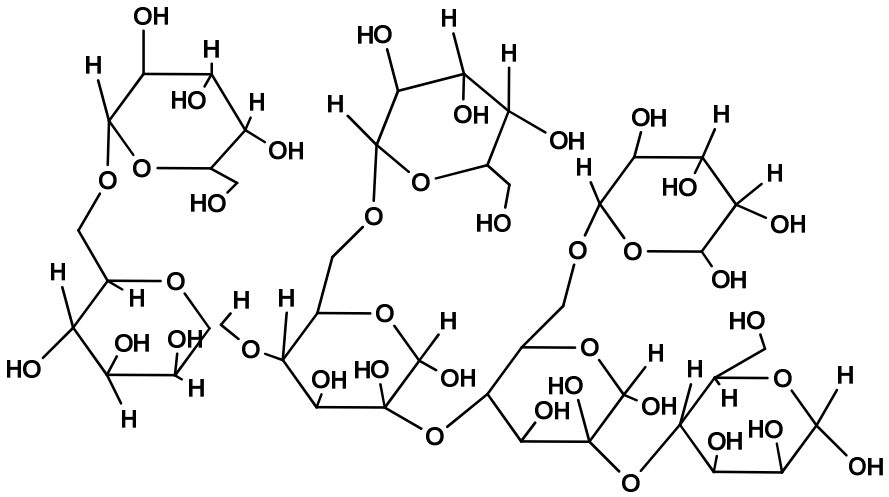

Fig.6. Structure of gum ghatti.

However, because of its inconsistent quality and wide variation in its viscosity from batch to batch, it could never become established as a major tree-gum [86]. "Gatifolia," a new gum ghatti product, has been produced by a non-chemical, physical process that involves dissolving, filtering, sterilization and spray-drying. It has been claimed to have superior emulsification ability, acid resistance and salt tolerance [87]. The gum has a minimum viscosity but it extremely soluble in water as compared to other natural gum.

1.2.7. Gum acacia

Dietary fibrous food grade gum acacia is a hetero natural polysaccharide produced from A. *seyal* and *Acacia Senegal* trees, and highly used food hydrocolloids [88]. The molecular weight of gum acacia is varying from 350-850 kDa and contain 44 % galactose, 27 % arabinose residues, 13 % rhamnose 16 % glucuronic (Fig. 7) and also has some minerals like K, Ca and Mg [89]. Gum acacia has a vital role in industrial manufacturing, spanning from food, pharmaceutics, paint, textile, printing industries [90]. It has proved itself indispensable as a stabilizer, emulsifier, bulking agent, shelf-life enhancer, encapsulating agent for bioactive components, satiating agent. Its anti-inflammatory effects on multiple organs have elevated its status as a safe food additive.

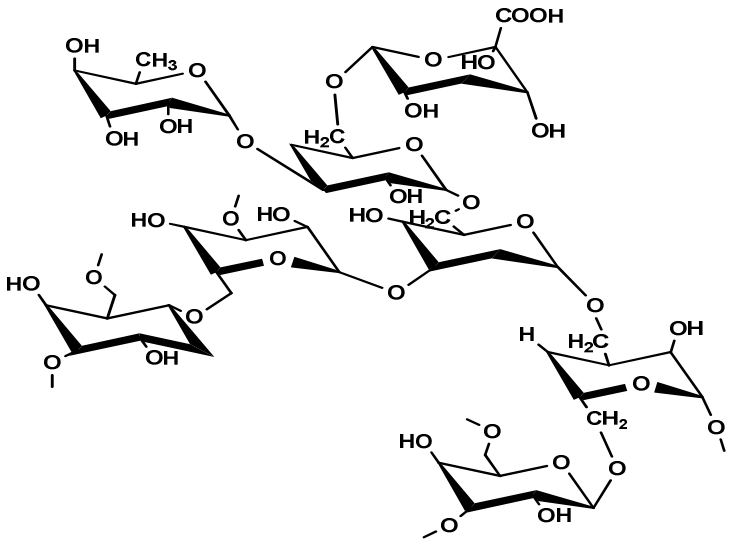

Fig.7. Structure of gum acacia.

Now, gum acacia is increasingly being implicated in nano-medicines and biosensors. Among other effective uses, controlled drug delivery and better bio-dispersion of nanoparticles are most pronounced [91].

1.2.8. Starch

Starch is most abundant cheap, biodegradable, renewable and natural polysaccharide. Starch products have applications in many industries, like food, textile, paper, plastic, adhesives, cosmetics and pharmaceutical industries. Starch is the main part of foods and initial material for industrial production. Starch is found in numerous parts of plants such as leaves, fruits, seeds, stem and roots. Wheat, potato, cassava, banana, rice, corn, etc., are also rich sources of starch. Starch is mainly composed of two homopolymers of D-glucose; amylase and amylopectin. Amylase is mostly linear α-D (1→4) glucan and amylopectin has a backbone structure as amylose but with many α-(1→6) linked branch points. Starch has different proportions of amylose and amylopectin ranging from 10–20% [92] and amylose and 80–90% amylopectin depending on source [93, 94]. Starch occurs naturally as discrete granules since the short branched amylopectin chains are able to form helical structures which crystallize. Starch granules exhibit hydrophilic properties and strong intermolecular association via hydrogen bonding formed by hydroxyl groups on the granule surface [95]. Linear (a) and crosslinked (b) structures of starch are shown in Fig.8. The available hydroxyl groups on the starch chains potentially exhibit reactivity specific for alcohols. In other words,

they can be oxidized and reduced and may participate in the formation of hydrogen bonds and ethers. Starch is a renewable, biodegradable, inexpensive, widely obtainable and environmentally friendly material [93].

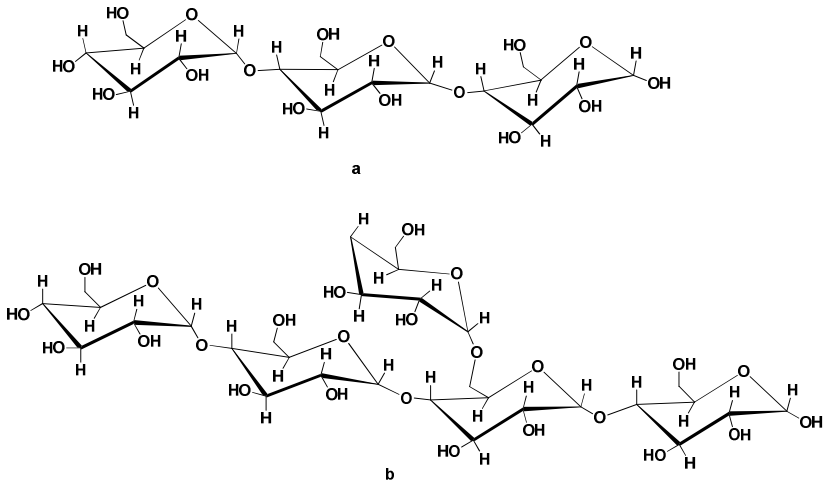

Fig.8. (a) Linear and (b) crosslinked structure of starch.

1.3.Method of modification of the polysaccharide

Polysaccharides are infrequently consumed in its intact form and normally used by industry in its native form. Polysaccharides have the limited application in their natural form because they are unstable with respect to pH, temperature, time and shear forces. Native polysaccharides show a high tendency for decomposition and retrogradation [96]. Additionally, some polysaccharides are inert, insoluble in water at room temperature, greatly resistant to enzymatic hydrolysis and consequently deficient in functional properties. Polysaccharides are often modified to develop specific properties such as texture, adhesion solubility, and tolerance to the heating temperatures used in industrial processes [97].

Several methods have been developed to produce modified polysaccharides with a variety of properties and applications. All of these techniques alter the natural polymer, making it greatly flexible and varying its physicochemical properties to increase its value for various applications. Grafting and crosslinking are the significant methods to modify the natural polysaccharides [5].

1.3.1. Graft copolymerization

The graft copolymerization is quite simple and very useful technique to modify the naturally occurring polysaccharide. The obtained grafted materials are demonstrating good resistance to heat or abrasion, greater mechanical strength, higher oil/water disgusting

qualities, or antimicrobial activity as compared to virgin polysaccharides. Pure polysaccharides have good resistant to degradation under the influence of shear and therefore, applicable for flocculants [98] but have small shelf life due to their vulnerability to biodegradation. The synthetic polysaccharide can be simply tailored, but they affect from weak shear resistant characteristics and can be converted into extremely customizable matrices with hybrid characteristics appropriate for various applications [99]. Chemical grafting is a significant technique to enhance the compatibility between natural polymers and synthetic to obtain novel materials with hybrid properties due to attachment of monomers, onto polymer backbone [100].

Various vinyl monomers, such as acrylic acid [101] acrylamide [102], methacrylamide[103], acrylonitrile [104], methyl methacrylate [105] and *N*-tertbutyl acrylamide [106], have been used for grafting onto various natural polysaccharides to increase their commercial application. The grafting of vinyl monomer increases the flocculating properties of the polysaccharides wherein grafted materials are capable to remove the pollutants easily [2]. Graft copolymers of the natural polymer are established and have applications in textile, petroleum, paper, pharmaceutical industries and also used for environmental protection [107].

Grafting of the suitable monomer is a significant procedure to add the novel properties in the natural polymer backbone without loss of native properties (Fig. 9). It is a chemical technique which adapts the desired features in natural polysaccharide without any change of their intrinsic behaviour i.e. grafting improves the symmetry of the polymeric chain, stability, both intra and inter molecular forces, crystallinity, glass transition, solubility, temperature, permeability, elasticity, and chemical reactivity of polysaccharides.

Many scientific communities have been modified the polysaccharide or natural polymer via grafting of small molecule (vinyl monomers) on to a backbone of polysaccharide or natural polymer in the presence of redox initiator. The process of grafting can be completed with or without the use of the redox initiator through conventional (thermal method) and non-conventional techniques [2]**.**

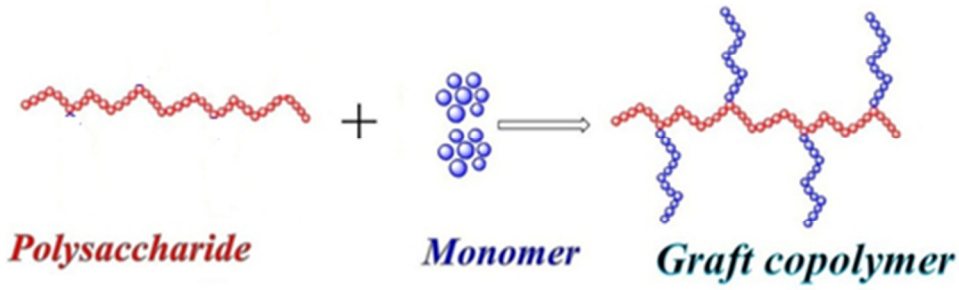

Fig.9. Structure of grafting.

1.3.2. Cross-linking copolymerization

Cross-linking is a stabilization technique in polymer chemistry which leads to the multidimensional extension of the polymer chain, resulting in the network structure [108]. Crosslinking is ionic or covalent bond which joins one polymer backbone to the other. Cross-linking changes a liquid polymer into solid or gel by restricting the ability of movement i.e. a liquid polymer can be turned into a solid or gel by cross-linking the chains together [109]. When polymer chains are linked together by cross-links, they lose some of their ability to move as individual polymer chains. Cross-linking increases the molecular mass of a polymer. Modifying the polymer molecular weight distribution through crosslinking is one of the simplest methods of achieving the desired properties. The crosslinking of polymers to form gels has been used for decades in the automotive industry to produces tires. More recently, polymer crosslinking has been applied to environmental clean-up, wound healing materials, consumer products, artificial organs, self-healing coatings, and the micro-patterning of surfaces [110, 111].

1.4.Methods of grafting

The polymer chains are grafted on polysaccharides through three main strategies, the 'grafting through', the 'grafting on' and the 'grafting from' processes. The grafting through process includes copolymerizing premade vinyl monomer with polysaccharide and the grafting from' (free radical) processes involving the progress of grafting directly on polysaccharide backbones and free radicals are easily generated on polysaccharide backbone by chemical initiators. A large number of synthetic methods of graft copolymerization have been developed. There are three main synthetic methods of graft copolymerization such as (I) conventional chemical method, (II) microwave methods and (III) enzyme method.

1.4.1. Grafting by conventional method

The conventional free radical processes are the 'grafting form' technique involving the growth of grafts directly from the polysaccharide backbones. It is the most significant and useful technique. Conventional grafting procedures may lead to polysaccharide backbone degradation and are not susceptible to block copolymer formation. Their use may often be harmful to some applications as they have limited control over graft molecular weight distribution. These problems have been addressed through the use of controlled/living radical polymerizations to obtain graft-functionalized polysaccharide based macromolecular materials [112].

The preparation of grafted compound depends on the free radicals which are obtained in situ through redox initiators such as ferrous ammonium sulfate, ethylene diamine tetraacetic acid, potassium persulfate, ceric ammonium nitrate, ferrous ammonium sulfate/potassium persulfate and $K_2S_2O_8$/ascorbic acid (Fig. 10).

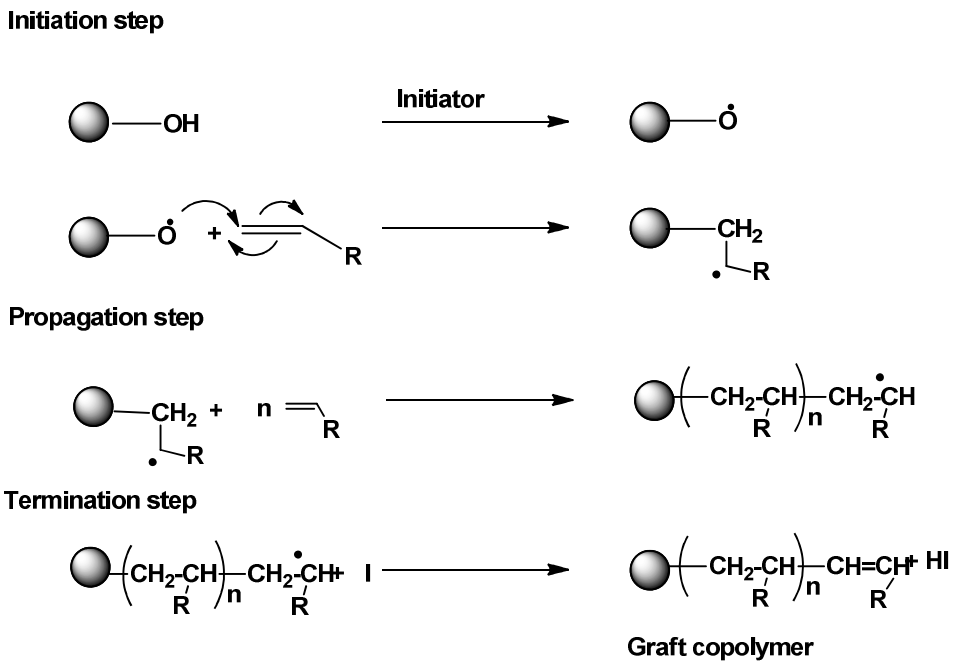

Fig.10. Scheme of free radical grafting.

Modification of polysaccharide via free radical initiated graft copolymerization is a very useful process for the industrial application of polysaccharides. However, these transformations generally require inert conditions and are tedious as well as time taking processes. In conventional processes, the copolymer materials are often accompanied by the

formation of homopolymers and the reduction in yield results in contamination of the copolymer product. Apart from the use of radical initiators, free radicals are generated by ultraviolet radiation, electron beams and microwave irradiation. Conventional grafting methods cause degradation of the polysaccharide backbone and cannot inhibit copolymer formation. Their use can often be detrimental for some applications due to limited control over the graft molecular weight distribution. These problems are addressed by the use of controlled/free radical polymerization to obtain grafted polymeric materials based polysaccharide. Said et al synthesized the grafted chitosan [chitosan-g-(acrylic acid-co-acrylamide)] through the grafting in presence of potassium persulfate free radical initiator and the mechanism of synthesize is given in Fig. 11 [113].

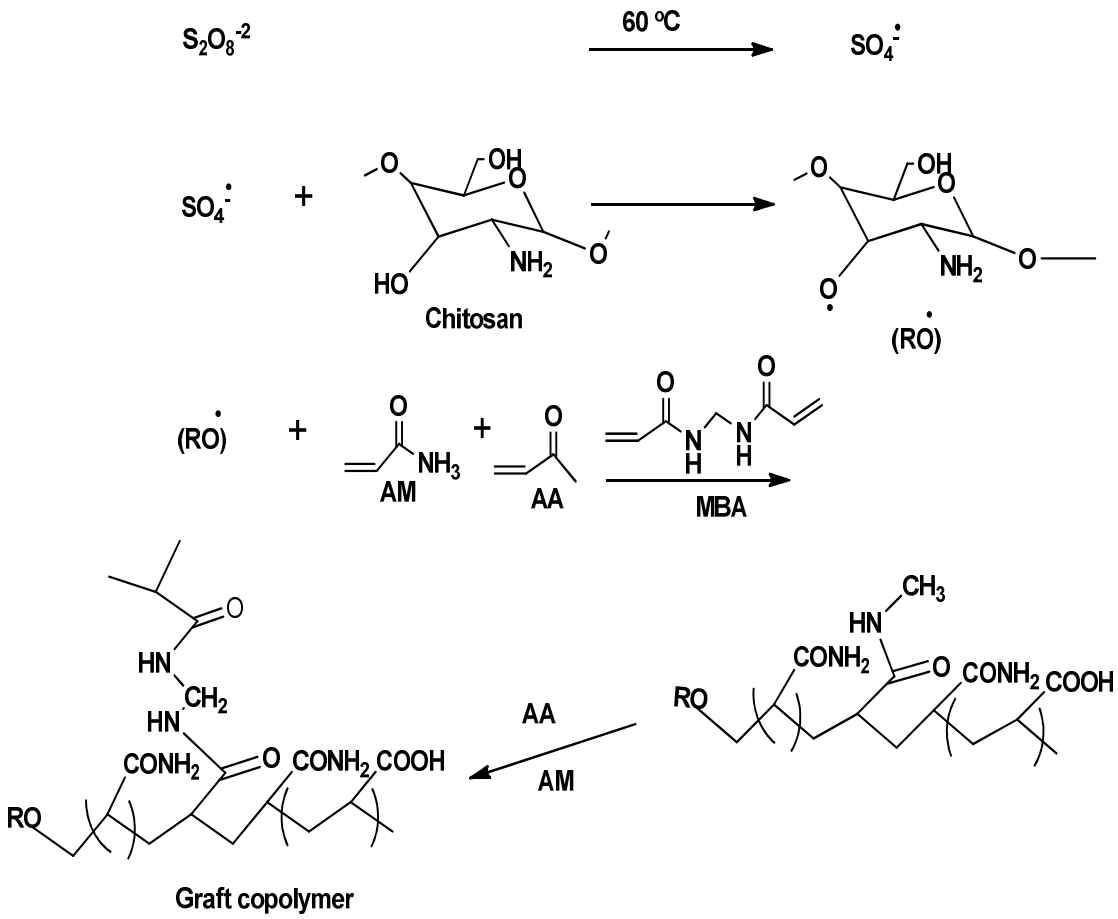

Fig.11. Scheme of synthesis of chitosan -g- (acrylic acid-co-acrylamide).

Abdul-Raheim et al. prepared the starch-g-acrylic acid in presence of benzyl peroxide for removal of some poisonous metals Fig. 12 [114].

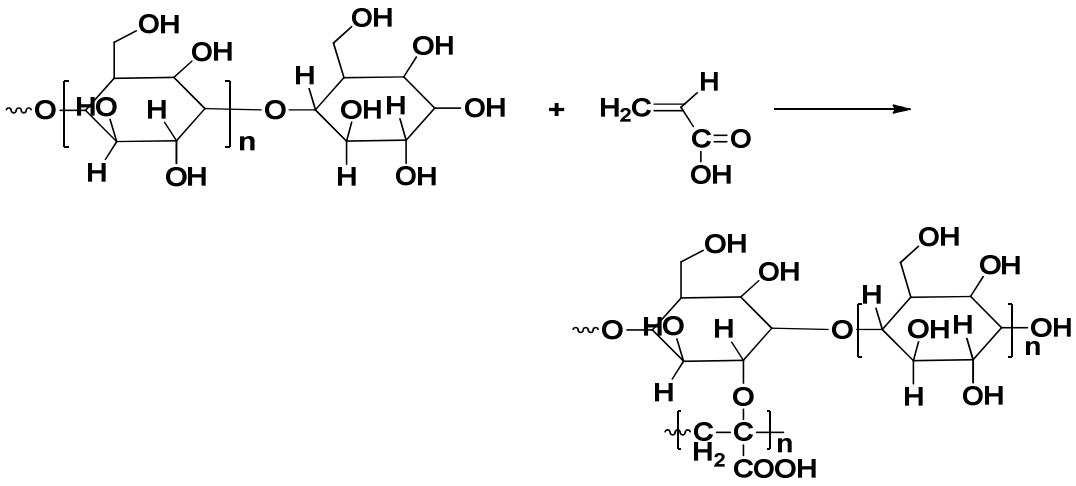

Fig.12. Scheme of synthesis of starch-g- acrylic acid.

1.4.2. Grafting by microwave (MW) irradiation.

Modification of polysaccharides via microwave radiation is a better technique of grafting as a comparison to conventional method or other chemical methods and demonstrates higher efficiency of grafting, greater percentage yield, low time consumption, low cost and greater thermal stability. Microwave radiation technique is one of the clean, green and eco-friendly technique of modification of polysaccharides and the future prospective of microwave radiation technique would be important influences to modify the natural polymer general mechanism given in Fig. 13.

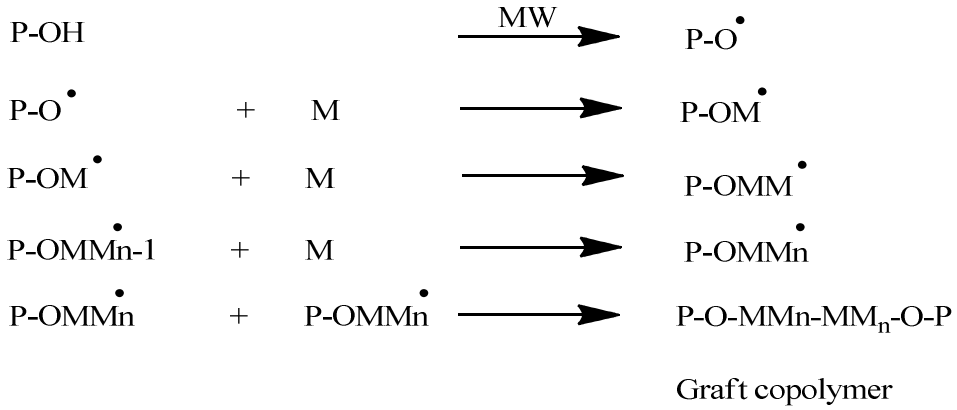

Fig.13. Scheme of synthesis of graft copolymer by microwave initiator.

An inert atmosphere and high quantity of solvent required to perform the conventional thermal grafting reactions while grafting reaction under microwave irradiation can be carried out in open reaction vessels with minimum solvent. The enhancement of physicochemical

properties to which the compounds are exposed during the conventional methods is also decreased under microwaves. Moreover, the use of microwaves enhances the percentage yield of grafting in very short reaction time as compared to general heating methods. In order to control the yield of the graft, the microwave power and the irradiation time are electronically controlled to ensure accurate control and reproducibility of grafting yield. The variance in compound selectivity can be attributed to the different types of heating in the two processes. Microwave heating has great influence and the heating is a significance of the dielectric loss in the irradiated medium resulting in almost immediate homogeneous heating of compound in a selective mode which results higher heating rates and improved product selectivity [115]. Polyacrylamide grafted Psyllium (Psy-g- PAM) was synthesized through the microwave process and the mechanism given in Fig. 14 [116].

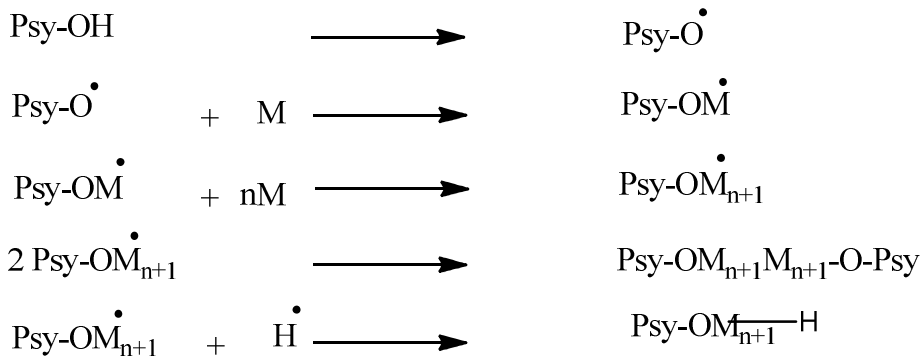

Fig.14. Scheme of synthesis of Psy-g-PAM.

Gum acacia-graft-polyaniline was synthesized according to the following mechanism through the microwave method reported by V. Singh et al. (Fig. 15) [117].

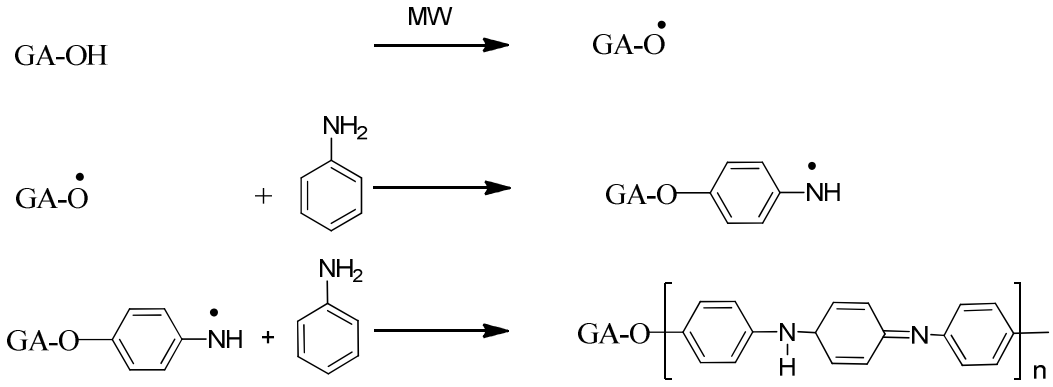

Fig.15. Scheme of synthesis of Gum acacia-graft-polyaniline.

1.4.3. Enzymatic grafting

The synthesis and modification of polymer through enzymes are very beneficial to the purpose of health and safety because it eliminates the hazards materials which linked with reactive monomers. Enzymes are also used for environmental beneficial because it decomposed the waste into simple elements and also eliminate the hazardous material.

Finally, enzyme specificity may provide the possibility to accurately modify the polymer structure without loss of any polymer function. Many chitosan derivatives have been synthesized through enzymatic modification which improves some unique properties of chitosan like, water solubility, thermal stability, pH sensitive and adhesive [118] properties. Enzymatic modification of natural chitosan with synthetic phenolic compounds to increase water solubility in basic environments reported as (Fig. 16) [119].

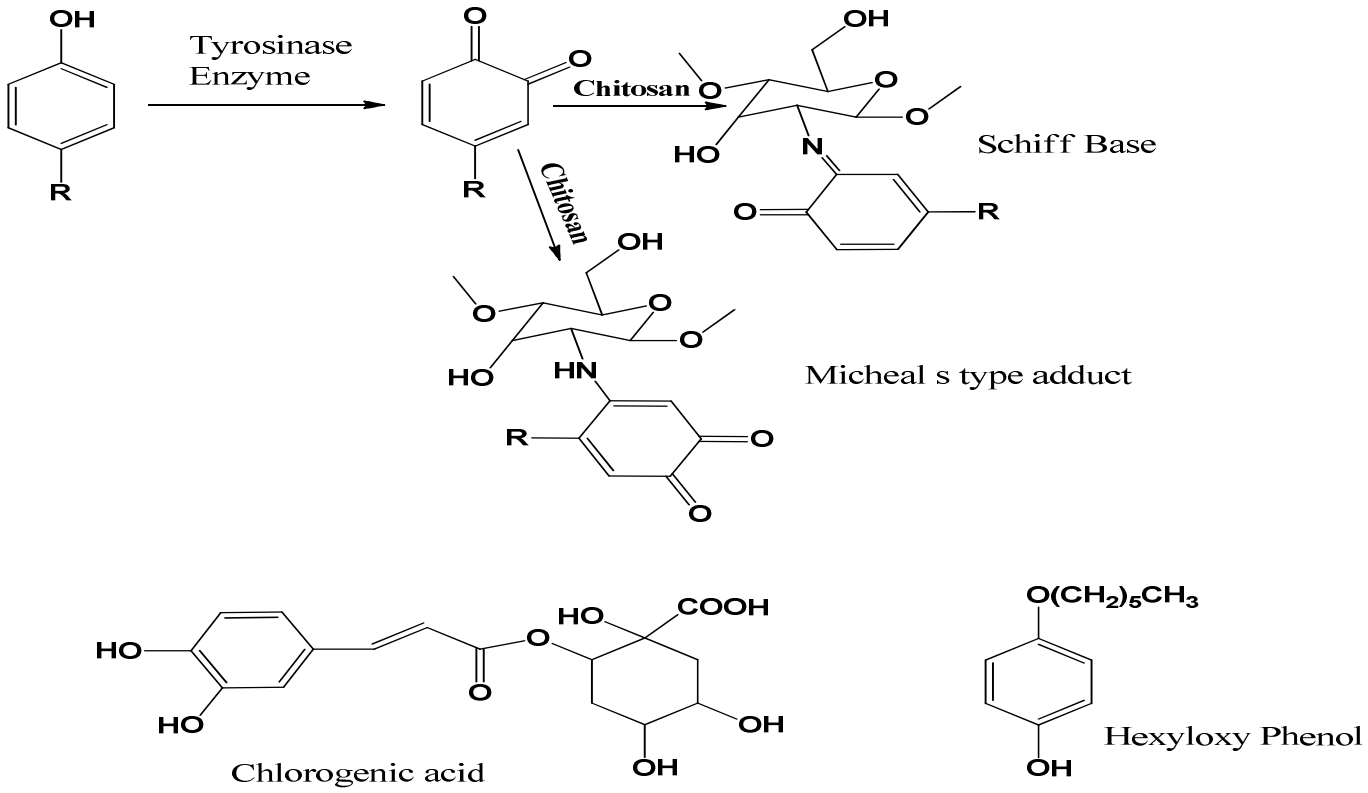

Fig.16. Enzymatic grafting of chitosan with phenol and tyrosinase.

1.5.Characterization techniques of grafted and crosslinked polysaccharides

1.5.1. Fourier-transform infrared spectroscopy (FTIR)

The use of Fourier Transform infrared spectroscopy for the characterization of the polymeric materials is very increasing in recent years, because a variety of sampling techniques are available but Fourier Transform infrared spectroscopy is the most significant technique to identify the kinds of functional group of polymeric materials and chemical bonds in compound via generating infrared absorption spectrum which is like to fingerprint of molecules [120] and also used for identifying the materials are either inorganic or organic. It is also utilized to quantitate some constituents of an unknown complex and for the characterization of gases, liquids and solids [121]. FTIR generates the absorption bands in IR region of the spectrum due to the interaction between electromagnetic radiation and molecular vibrations. Chemical bonds vibrate at many frequencies which depend upon the compounds and types of bonds. For any bond, there are several suitable frequencies at which bond can vibrate. According to quantum mechanism, this frequency corresponds to the ground state and many excited states. One way to increase the frequency of a molecular vibration is to stimulate binding by absorbing light energy [122]. In this research work, FT-IR spectra were obtained by Fourier transform infrared (FTIR) (Nicole-6700). FTIR spectrum provides the necessary information about the molecular structure such as functional group and chemical bonding in the region 4000-450 cm^{-1}. All samples were kept away from light and dried for 1 hour at 50^{o}C temperature in an oven under reduced pressure. The dried samples were ground with KBr and prepared the KBr pellets containing 1 % (w/w) of samples before analysis.

1.5.2. Scanning Electron Microscopy (SEM)

The scanning electron microscopy is an advanced version of electron microscopy [123] which provides evidence about the crystalline structure, surface topography, chemical composition and electrical behaviour of materials by scanning it in raster scan manner with a high-energy electrons beam. The electrons beams interact with the atoms of sample generating signals which produced high resolution (20nm to 1nm) images of the sample surface and also provide some other information such as electrical conductivity and composition [124]. SEM images have a characteristics 3-D appearance and therefore useful to evaluating the external structure of the sample. The primary electrons generating from the

surface of the source are elastically dispersed via atoms in the material. The electrons emitted are detected to produce an image. Beside the emitted electrons, X-rays also obtained through the interaction between the sample and electrons. These can be identified in SEM prepared for energy dispersive X-ray (EDX) spectroscopy [125, 126]. In this research work, the surface morphology of polymeric materials was studied by scanning electron microscope (JSM, 6490) with an accelerating voltage of 15 kV.

1.5.3. X-ray diffraction (XRD)

X-ray diffraction (XRD) is a significant analytical, non-destructive method that is used mostly for characterizing crystalline substance and gives information about the structures, phases, crystal arrangement and some other essential parameters, like strain, crystal defects, crystallinity and regular grain size [127]. In this research work, X-Ray Diffraction analysis was carried out on Bruker-D8 advance diffractometer (Shimadzu, Japan) in 2-theta angle range 5^o-75^o with a steep angle of 6^o/min. X-ray diffraction spectra are obtained via interference of X-rays (monochromatic beam) scattered at exact angles from every set of lattice planes in substance. The intensity of peak is measured through the distribution of particles inside the lattice. Therefore, the X-ray diffraction configuration is the fingerprint of material in which particles arranged [128].

The interaction between the incident light and the sample produces constructive interference (and diffracted light) if the condition satisfies the Bragg's law (eq. 1.).

$$n\lambda = 2d \sin \theta \text{ ……..(1)}$$

Bragg's law is a relationship between wavelengths of electromagnetic radiation, inter planar spacing and diffraction angles of crystal sample and are then detected, treated, and counted. By scanning the material with 2θ angles, all probable diffraction of the lattice [129] should be achieved due to the random arrangement of the ground material. Transformation of the peak of diffraction into d-spacing permits the identification of the material since every sample has a group of individual d-spacing.

Typically, this is achieved by comparing the d-interval to a standard reference pattern. The diffract gram of semi-crystalline or amorphous sample are like broad humps. The degree of crystallinity of materials is quantitatively estimated. The Degree of crystallites of the sample is very small the peaks will be broad. The degree of crystallinity is calculated by following equation (2) [130].

$$Xc = \frac{\text{Ac}}{(\text{Ac}+\text{Aa})} \text{.....(2)}$$

Where

Xc = degree of crystallinity.

Ac = crystallized segment.

Aa = amorphous segment.

The X-ray diffraction machine is made of three main parts (I) an X-ray tube, (II) a sample holder, and (III) X-ray detector[131].

1.5.4. Thermal gravimetric analysis (TGA)

In this method thermo-balance works that continuously determined weight change of sample with respect to temperature in the N_2/inert environment. TGA is generally working with respect to nanocomposite, hybrid materials and polymeric materials to investigate the thermal stability, amount of inorganic components, which usually stays until the end of the measurement due to its thermal stability, and the level of absorbed moisture or organic volatiles in these materials. Typical TGA plots show the weight loss in relation to the temperature and typical range that can be distinguished are the loss of moisture and adsorbed solvent up to 150 °C, the decomposition of organic compounds between 200 to 600 °C. The observed weight loss in the polymer can be the result of volatile products formed by thermal degradation. The gravimetric estimation of moisture, volatile ingredients and inert or thermally stable additives in a polymer can be easily made by this technique. In this research work, Thermogravimetric analysis (TGA) was performed using SII 6300 EXSTAR TG-DTA (Japan) and all measurements were carried out under a nitrogen atmosphere.

1.5.5. Differential scanning calorimetric

DSC is a significant technique for thermal analysis and measures the variance in the amount of required heat to increase the temperature of reference and sample. During the experiment, the reference and sample are maintained at the same temperature. In general, the temperature for DSC analysis is planned that temperature sample holder enhances linearly in function of time. An endothermic and exothermic event in the material results in a deviation [132] in the difference of between the two heat flows to the reference and result in a peak in

the DSC curve. The difference in heat flow between the sample and reference also delivers the quantitative amount of energy absorbed or released during such transitions. This information can be obtained by integrating the peak and comparing it with a given transition of a known sample and the experiments can be carried out under nitrogen, oxygen and another inert gas atmosphere.

1.5.6. UV- Visible spectroscopy

UV-Vis Spectroscopy is a significant analytical technique used to determine the absorbance of visible or UV radiation via an analyze. UV radiation involved with electronic excitation, when a molecule having the π electrons, absorb the UV-Vis radiation light, then valence electrons become excited and exhibit a transition from ground state to higher. It is an effective method for both quantitative and qualitative analysis of inorganic and organic materials. This technique is most often used in a quantitative way to measure the concentration of absorptive specimens using Beer-Lambert's law (eq. 3).

$$A = -\log_{10}(I/I_0) = \in CL \text{…….(3)}$$

Where

A = Absorbance, I_0 = intensity of the incident light at a given wavelength, I = transmitted intensity, L = path length in sample, and C = concentration of the absorbing materials, ε = constant (extinction coefficient or molar absorptivity). UV –Visible studies on the different samples throughout the work was carried out by using Shimadzu UV –Visible spectrometer model UV 2100, Kyoto, Japan.

1.6. Application of graft and crosslink copolymers

1.6.1. In Drug delivery

Polymeric materials are more comfortable and safe with drugs as a carrier in drug delivery systems due to their pulsated release of drug at desired temperature and pH for targeting site. Considering these impressive polymeric properties, preferred polysaccharides, which are a biological active natural polymer [133] and possess very interesting biological properties, namely nontoxicity [134], biodegradability [135], biocompatibility, cytocompatibility [70], antimicrobial [71], antioxidant, anti-cholesterolemic [72], anti-inflammatory, analgestic [73], haemostatic, mucoadhesion [74]. Therefore, polysaccharides

have been used in a variety of applications, most relevant in the medical as well as pharmaceutical fields [75]. On the other hand, polysaccharides were not shown considerable applications in drug delivery, due to its solubility. To resolve this problem, graft copolymerization is the best option to modify the native property of pure chitosan for drug delivery application through the microwave technique. Grafted/cross-link derivatives of polysaccharides have been highly used as drug delivery carrier for the controlled release of oral drug by virtue of their non-toxic, biodegradable, and biocompatible behaviour.

Recently, several advanced polysaccharide based drug delivery devices have been established for drug delivering application [136], to boost the technique advancement in the field of biomedical applications [137]. Although, many researchers and pharmaceutical industries developed a more sophisticated and effective drug carrier which releases the drug molecules at the right time and in desired amount. Therefore, the drug molecules reach their target on the right way with considerable concentration [138]. The high demand for cheaper and effective therapies, research and developments have been formulated new and advanced drug delivery systems for years [139].

Psyllium and chitosan are highly used as an active agent in drugs and biologics due to its physicochemical [140] and biological properties. Chitosan is hydro-soluble and positively charged polysaccharide and this property allows it to interact with the negative charged materials, polyanions and polymer. Chitosan has significant biopharmaceutical properties [141] such as biocompatibility, low toxicity and pH sensitivity. Because of these favourable characteristics, interest in chitosan and its derivatives as excipients in drug delivery has enhanced in the present time. Parsian et al. investigated that targeted delivery of anticancer agents' increases efficacy while reducing harmful effects. Among several drug delivery devices, nanoparticles of iron oxide coated by chitosan (CsMNP) attracted attention due to their biodegradability, low toxicity biocompatibility, and target orientation under magnetic area [142]. Alam et al. prepared the mucoadhesive based microparticles of furazolidone (antimicrobial agent) for the stomach target drug delivery system by using the chitosan as mucin adsorptive polysaccharides via spray drying method [143]. Balan et al. synthesized the nano drug carrier (biotinylated *N*-palmitoyl chitosan) for the treatment of breast cancer. They found that nanoparticles demonstrated a better drug loading capability and susceptibility to hemocompatibility, biodegradability and pH-dependent drug release behaviour the properties

that can be utilized in drug delivery applications. [144]. Huo et al. prepared the chitosan-microcapsules/starch blend film based drug delivery device, for controlled antofloxacin (antibacterial) drug release and the drug releasing mechanisms was pH-sensitive [145].

Psyllium also used as a suitable drug candidate for controlled the drug release or controlled release devices and also used as therapeutic targeted devices. Singh and their co-workers have been widely used modified psyllium (hydrogel) in various types of drug delivery systems [146].

Bhatia et al. synthesized the anionic carboxymethylated psyllium and tested it for preparing the nanoparticulate drug carrier with polyelectrolyte complex and chitosan [147]. Cavallari et al. prepared the mucoadhesive patches of psyllium to the controlled release of chlorhexidine for pathologies of the oral cavity through casting solvent evaporation method [148].

Singh et al. prepared the psyllium and polyacrylamide based material in presence *N, N*-methylenebisacrylamide. They studied the swelling behaviour of hydrogel and also found the psyllium based hydrogel was pH dependent and have application in colon-specific drug delivery. They also prepared the drug delivery system based on psyllium-NVP via radiation polymerization and observe that hydrogel of psyllium [Psy-cl-poly(AA)] has wide application for colon specific drug delivery [149].

1.6.2. Waste water treatment/water purification

Continuously increasing water pollution is a very serious concern for the entire animal kingdom. All the animals, plants and human being are directly or indirectly affected by discharging of industrial, domestic and medical wastes along with agricultural effluents into the rivers and groundwater, which disturb the biological balance of aquatic system. A huge population is not able to get safe, clean and pure drinking water [150, 151]. Water pollution became a serious issue in industrialized regions. Textile industries, paper industries, pharmaceuticals, tannery, bleaching industries and other metal processing have major role to pollute the water resources. These toxic metals ions and dyes are not only potential human health hazards but also hazardous for plants as well as for aquatic life, especially dyes as these remain unchanged in the environment for long period [152]. Many natural polysaccharide adsorbent derivatives have been prepared for adsorbing metal ions and dyes by grafting novel functional groups on the polysaccharide chain and these functional groups are combined with polysaccharide to enhance the sorption capability and change the pH value

for metal and dye sorption via transforming the sorption surface in order to enhance sorption selectivity for the toxic metal.

Mucilage and gums have been suggested as safe substitutes for conservative polysaccharide in wastewater treatment for their manufacturing procedure and applications which are environmental friendly and valuable to human and ecology. Up to date, natural flocculants based on gums and mucilage that is derived from plant species including Okra, Mallow, Psyllium, Tamarind and Fenugreek has been developed. These natural polysaccharide based flocculants are mostly prepared via aqueous extraction and precipitation with methyl alcohol and drying [153, 154]. Thombare et al prepared the novel adsorbents for water refining; hydrogels of guar gum and borax via crosslinking process and its efficiency to removal of aniline blue dye from its aqueous solution was also tested. More than 90% removal of dye was achieved by using the 1.0 g hydrogel in 60 minutes [155]. Bandyo-padhyay et al. prepared the gaur gum and acrylic acid based graft copolymer (GG-g-PAA) through surfactant mediated free radical polymerization technique for wastewater treatment (adsorption of lead from contaminated water) and maximum adsorption (89.62%) was achieved at pH of 4.5 [156]. The beads of chitosan gel show high water content capacity and poor volumetric density of sorbent sites. The grafting of amine groups on chitosan can offset this disadvantage [157].

1.6.2.1. Adsorption of metal ions

Toxic metals, like lead, tin, mercury, selenium, arsenic and cadmium are introduced to the water/soil by various human activities and get deposited gradually in the ecosystem. The drainage of polluted water into rivers and lakes is very common [158]. Reaching of the toxic metals into fresh water resources are causes poisoning of fresh water resources which influences the entire eco-system due to uncontrolled human activities. Highly toxic metals with their permissible limits (as per WHO), toxic impact and major sources are given in Table-1. [159]**.**

Table-1:- Toxic metals with their permissible limits, toxic impact, and major sources.

S.N.	Metal	Toxicity Effect	Source	Permission level (mg/L)
1.	Mercury (Hg)	Irritation of respiratory system; lung, liver kidney damage, and loss of hearing and muscle coordination.	Pesticides, paper industry, batteries,	0.002
2.	Chromium (Cr)	Lung damage and Irritation or respiratory system	Paints, electro plating and metallurgy	0.05
3.	Arsenic (As)	Irritation of respiratory system, Liver and Kidney damage, Loss of appetite, nausea and vomiting etc.	Metal smelters. fungicides, Pesticides	0.020
4.	Lead (Pb)	Lung and liver damage; loss of appetite, nausea	Pesticide, automobile, emission, mining.	0.15
5.	Cadmium (Cd)	Lung, liver and kidney damage; Irritation of respiratory system	Welding, electroplating, pesticide fertilizer, Cd-Ni batteries.	0.06
6.	Nickel (Ni)	Lung, liver and kidney damage	Electrochemical industries	0.1

Removal of toxic metal from the aqueous solution is one of the great challenge and task for the scientific community in the last few years. Several natural polysaccharides and their products have been prepared for the purpose of adsorbing metal ions by grafting novel functional groups onto the polysaccharide backbone [160]. Tripathy et al. prepared the Starch-g-Poly-(N-methylacrylamide-co-acrylic acid) in presence of KPS initiator via grafting method. This grafted material was used as adsorbent for removal of mercuric ions from its water solution. The maximum adsorption was found at pH 5.5, contact time 180 min, temperature 45 °C and adsorbent dose was 0.014 g [151]. Sci et al. prepared a novel low-cost

chitosan-modified silicon material that adsorbs anionic heavy metals. Newly synthesized product has a broad absorption range of heavy metals in acid and alkaline media, a high adsorption efficiency of anionic Cr (VI) in solution and exhibit the major adsorption capacity at pH 5-6 [161].

Low cost, eco-friendly nano and biocomposite of zinc oxide with guar gum (GG/nZnO) was used to remove the Chromium metal from its solution and the greatest adsorption was achieved at 1.0 g/L adsorbent dose, 50 min contact time and 7.0 pH 25 mg/L Cr (VI) concentration [162, 163]. Lalita et al. synthesized the low cost and eco-friendly hydrogel for sorption of heavy metal ions via grafting and crosslinking of acrylic acid alone and with other molecule acrylonitrile, glycidyl methacrylate and acrylamide onto chitosan. They also observed that modified chitosan showed good results compare to pure chitosan and showed favored sorption of Fe(II) ions over Cr(VI) and Cu(II) ions [164]. Crosslink material, based on guar gum was synthesized via grafting of acrylamide onto guar gum, in presence of potassium bromate/thiourea dioxide initiator. This material was treated with glutaraldehyde to obtain the sorbent hydrogel. The hydrogel was used to remove the chromium metal from aqueous solution [165].

1.6.2.2. Dyes removal

Dyes are one of the great threats to aquatic system and our environment because low concentrations of dyes are extremely visible (aesthetic pollution) and disturb the aquatic life and food chain (chemical pollution). Dyes are anionic, cationic, and non-ionic coloured stable compounds which obtained from the natural product without treatment [166] such as plants, beetles, animals [167] and minerals and widely used in textiles, cosmetics, printing, rubber, plastics, leather industries [168] to colour their products. Therefore large amount of dyes (colour compounds) reached in water sources with industrial wastewater and become great cause of water pollution. Dyes are highly toxic, carcinogenic, stable, non-biological degradable coloured compounds and very danger for human health, animals and aquatic life and remain unchanged for long time; therefore, there is an urgent need to remove dyes from dye affected water bodies. Polysaccharide-based adsorbent highly used for the removal of dye from their solution [169]. Chitosan has found wide application in water treatment. The functional side group of chitosan makes it a promising adsorbent for contaminants (e.g. synthetic dyes) by electrostatic force and hydrogen bonding [170]. Chitosan has potential as an efficient adsorbent for almost all kinds of dyes other than the basic pigment, which can be

directly related to its natural cationic properties. There are several reports on chemical modification of chitosan to remove simple dyes from coloured fibre [171]. The chemical modification of chitosan via grafting of poly (acrylic acid) and poly (acrylamide) in presence of persulfate redox initiator through grafting and covalent cross-linking processes for removal of simple dyes and the maximum adsorption capability was reported by Lazaridis [172, 173].

Abbasian et al. synthesized the series of grafted chitosan derivatives such as chitosan-*g*-polyaniline, chitosan-*g*-poly(*N*-ethylaniline) and chitosan-*g*-poly(*N*-methylaniline) by chemical oxidation process for adsorption of acid red 3 and direct red 23 dye from aqueous solution [170]. Kiakhani et al. prepared the biopolymer adsorbent by grafting of chitosan with ethyl acrylate for the adsorption of Basic Blue 41 (BB41) as well as Basic Red 18 (BR18) from their solutions. The Chit-g-Ea has the higher adsorption ability toward simple dyes were 217.39 ppm and 158.73 ppm for BB41 and BR18, respectively [171].

Mishra et al. have improved psyllium through graft copolymerization with acrylonitrile and acrylamide using CAN/HNO_3 initiator in N_2 environment for its utility in flocculent for the elimination of poisonous wastes from tannery, sewage and textile wastewater [174, 175]. Chaudhary et al. synthesized a novel adsorbent of xanthan gum-psyllium with acrylic acid an itaconic acid by graft copolymerization for removal of cationic and ionic dyes from its solutions [176]. Grafting and crosslinking formation of psyllium with graft methacrylic acid have been described for water treatment by Kumar and Verma [177].

1.6.3. Food industries

Packaging is generally used to maintain the quality and extend the shelf life of food products [178]. The materials used for food packaging material consist of a variety of plastics, glass, metals, paper and cardboard. These materials provide physical protection and create proper physicochemical conditions for products that are essential for obtaining a satisfactory shelf life and maintaining food quality and safety. However, after their useful life, they cause a serious environmental problem since they are not easily biodegraded [179]. The solution of this issue is the use of biopolymers especially polysaccharides [180]. The applications of polysaccharide-based films in food products could offer new opportunities to develop novel food packaging systems. Also, biodegradable films can reduce environmental problems associated with food packaging [180].

Several studies have been reported that polysaccharides (cellulose, chitosan, starch, pectin, alginate and carrageenan) were used to syntheses the edible films for food packaging (Table- 2) [181]. Grafted chitosan films are an interesting agent in food conservation applications and as antimicrobial candidate in the various industries [182]. Regarding in food preservation field, there is a possibility of using chitosan to string then the preservation of fish during storage, to improve the quality of fresh broccoli, to prevent spoilage of cold pork products and to control bacterial contamination during brewing [183-186]. Many examples are known in which edible chitosan coating increase shelf life and maintains the nutritional and quality of vegetables and fresh fruits[187, 188]. Binary grafted chitosan film [chit-g-Poly (An-*co*-Am)] with acrylamide and acrylonitrile was protected apple and guava from microbial attack and extend their shelf life and is biodegradable in nature.

Table-2:- Properties and food applications of polysaccharide-based film

Polysaccharide	**Composition**	**Properties**	**Main Food Applications**	**Refs**
Chitin	*N*-acetylglucosamine	Biodegradable, antibacterial and fungi-static properties; bio compatible and non-toxic highly transparent	Coffee capsules food bags packaging films	[150, 152, 189]
Chitosan	D-glucosamine *N*-acetyl-D-glucosamine	Biodegradable biocompatible and non-toxic antifungal and anti- bacterial properties; good mechanical properties barrier to gases high water vapour permeability, brittle—need to use plasticizer	Edible membranes and coatings (straw- berries, cherries, mango, guava, among others) packaging membranes for vegetables and fruit	[189, 190]
Starch	Glucose	Biodegradable Transparent Odourless and tasteless Retro gradation high elongation and	Flexible packaging, (extruded bags, Nets for fresh fruit and	[191]

		tensile strength	vegetables) Rigid packaging Thermoformed trays and containers for packaging fresh food	
Galactom annans	Mannose Galactose	Biodegradable edible Semi permeable barrier to gases	Edible membranes and coatins (fruits, cheese)	[192]
Cellulose	Glucose	Biodegradable Good mechanical properties. Transparent Highly sensitive to water Resistance to fats and oils Need to perform modification, use of plasticizer or polymer blend	Cellophane membranes	[193]
Carrageenan	Galactose	Biodegradable, fragile and ductile behaviour, usually blended with other polymers	Coatings fruits meet encapsulation of aroma compounds	
Alginate	Mannuronic Glucuronic acid	Biodegradable. high water vapour permeability poor water resistance strong and brittle membranes cross-link with calcium	Coatings prevent water loss in fresh cut fruit (apple, papaya, pear and melon) inhibition of microbial growth (turkey products) microwaveable food (increase warming efficiency)	[193]

Xanthan gum	Glucuronic acid	Biodegradable, Edible	Edible coating 1.Meet (Prevent moisture migration during frying) 2.Fruit (Extend shelf-life)	[194]
Gellan gum	Glucose Rhamnose Glucuronic acid	Biodegradable Edible Lipid barrier Excellent gas barrier Good tensile strength	Edible Coatings in breading and batters for chicken, fish, cheese, vegetables and potatoes. Encapsulation of flavour and bioactive ingredients	[195, 196]

1.6.4. As antimicrobial agents

Microbial infections are serious issue because it is one of the leading cause of death worldwide, especially in medical institutions, where public are mostly more vulnerable [197] because the persistence of pathogenic microbial (bacteria, viruses and fungi) in various places, such as healthcare products, textiles, medical equipment, sanitary facilities and water purification equipment, because prevention of microbial infections are very difficult [198]. Natural polysaccharide represents significant types of materials with interesting characteristics that make them proper candidates in various utilities ranging from the bacteriological to biomedical fields. Natural polysaccharides with antibacterial activity are applicable for the direct removal of bacteria and as serve antibiotics. Regarding these issues, scientific community prepared the antimicrobial macromolecules for combat multi-drug resistant microorganisms. The use of polysaccharide as antimicrobial candidate generally shows various benefits since it has low long-term activity and low residual toxicity, chemically stable, non-volatile and does not penetrate via skin [199].

Polysaccharides with intrinsic antibacterial activity are generally based on polycations, that are capable to kill the microorganisms through act on their negatively cell membrane [9]. Generally, cationic groups present in these polysaccharides are quaternary phosphonium, quaternary ammonium, tertiary sulfonium or guanidinium [200, 201].

According to previously reported literature many natural polysaccharides, gums and mucilage exhibit the antimicrobial properties, among of that chitosan show the more probable antimicrobial properties.

Chitosan has a broad antimicrobial range to which fungi [202], gram-positive [203], gram-negative bacteria [204] are greatly susceptible. Chitosan and chitin have been examined as antimicrobial natural polysaccharide against bacteria, algae, fungi and yeasts in tests involving *in vitro* and *in vivo* interactions with chitosan in various forms (films, composites and solution) [205]. Usually, in these investigations, the chitosan is considered as a bactericidal or bacteriostatic. Goy et al, tested the antimicrobial property of chitosan and trimethyl chitosan, against Gram positive and Gram-negative bacteria via turbidity and well inhibition zone methods and found that chitosan was more active against the S. aureus (Gram-positive) than E. coli. (Gram-negative) [204].

Many articles explain the antimicrobial property of chitosan-based nanoparticles against E. coli, S. aureus. For S. aureus, nanoparticles of chitosan proved to have a minimum concentration of bactericidal (MBC = 4 g/ml) compared to pure chitosan (MBC = 32 g/ml). The antimicrobial activity was again improved when prepared nanoparticles of chitosan are loaded with Cu (MBC = 2 g/ml) [67]. Nanoparticles of chitosan was grafted with carvacrol or eugenol then exhibited antibacterial activity against S. aureus (MBC = 1 mg/ml) and (MBC = 2 mg/ml) respectively. The grafted samples were slightly more effective to the chitosan nanoparticles but less effective to the phenolic compounds [206].

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Chapter -2

Microwave assisted synthesis of binary grafted psyllium and its utility in anticancer formulation

A binary grafted copolymer of Psyllium mucilage (Psy) with acrylic acid (AA) and acrylonitrile (An) has been successfully synthesized under microwave conditions for *in vitro* drug release study. The grafting was confirmed by FTIR spectroscopy, XRD, SEM, EDX, TGA analytical techniques and the intrinsic viscosity studies. The swelling behavior of grafted material has been studied in solutions of different pH and time. We have also prepared Psy-g-Poly (AA-co-An) based beads with anti-cancer drug [(2-Chloro-3-(4-hydroxyphenylamino) naphthalene-1, 4-dione)]. The drug release behavior from Psy-g-Poly (AA-co-An) based beads has been determined in aqueous medium at different pH, where highest drug release was observed at pH 1.6. The drug release kinetics was analysed using different models. This study demonstrates that the release of drug depends on the composition of beads and pH of release medium. Kinetics of drug release from beads is best fitted by zero order and first order model.

2.1.Introduction

According to WHO reports cancer is one of the most serious health issue among human beings around the globe [1]. Colorectal cancer (CRC) is the third greatest dreaded diseases in the world and is the third main cause of cancer-related deaths. According to WHO report, by 2030 the worldwide growth rate of the colorectal cancer population will be 60% that means the new cases of cancer encountered will be about 2.2 million, and approximately 1.1 million deaths will occur in this period [2]. In many developed countries, change in habitual lifestyle and food habits result in a fast increase in both the colorectal cancer incidence and mortality [3]. The most common anti-cancer drug Capecitabine have very short plasma half-life (less than 0.85 h) leads to fast exclusion from human body, therefore need of this drug in regular high dose (approx. 1,250 mg/m per day) is required, but this high dose can responsible of over-dosage toxicities, including cardiotoxicity, bone-marrow depression, nausea, diarrhoea, vomiting steatites, and dermatitis [4]. To resolve these shortcomings, it is important to develop an effective and long-lasting acting targeted delivery system to reduce the high clearance rates of the drug and make it more effective, which is a challenging task in present scenario [5-7].

The colon-specific drug delivery system is that drug delivery system, which controls the drug release until drug reaches the cecum or colon. Therefore, proper action can be exerted into the diseased region without disturbing the normal region and cells of the upper gastrointestinal tract. This methodology facilitates the therapeutic efficiency of the medication, as well as minimizing hazardous or adverse effects [8, 9]. Most of the conventional drug delivery systems get failed in targeting colon because they are not able to carry the drug to the targeted site in the expected concentration at proper time. Designing this effective and safe colon targeting delivery system is the greatest challenging issue of the therapy [10].

In last few decades, natural polymers are widely used for the development of various drug delivery systems because they have several advantages over their synthetic counterparts such as cost-effectiveness, easy availability, biocompatibility and biodegradability. Several polysaccharides are widely used in the formation of drug delivery system [11]. Many research groups have been trying hard to modify the polysaccharide for developing specific,

novel and targeted drug delivery system with the purpose of better therapeutic efficacy, lesser side effects, and doses for the treatment of colon cancer [12].

Among the other polysaccharide derived drug delivery device, systems using psyllium have been broadly accepted as a probable site-specific with controlled drug delivery system for the colon-specific drugs [13, 14].

Psyllium is natural occurring fibrous food grade anionic polysaccharide widely used as a household medicine as well as drug delivery candidate for ancient time [15-17]. Psyllium is also known to reduce the risk of colon cancer [18] as well as tumorigenicity [19]. Psyllium fiber provides colonocytes some protection from deoxycholic acid-induced lysis. Propionic acid, a product of fiber breakdown, was a potent colonocyte mitogen, suggesting that fiber could indirectly protect the colon by providing colonocyte nutrients [20].

Psyllium is obtained from the seeds of the *Plantago ovate* plant [21]. It is also known as Ispaghula as well as Isabgol and made up by the combination of more substituted arabinoxylan polysaccharides [22]. Arabinoxylan is linear chains of xylose and arabinose units. Glucose, rhamnose, galactose and rhamnosyluronic acid residues are also present as minimum constituents [23-25]. The easy availability, low cost, non-toxicity, biodegradability and extensive usage since ancient times makes it a popular as well as natural choice to be explored further for various medicinal applications *viz.* constipation, diarrhoea, irritable bowel syndrome, ulcerative colitis, inflammatory bowel disease and colon cancer [26]. Drug delivery research based on psyllium has been highly conducted, to improve the diffusion rate of the encapsulated drugs by making polyelectrolyte complex between the hydroxyl and carboxyl groups. Psyllium based drug delivery system contacts with the mucus membrane by electrostatic force. The introduction of psyllium can improve bio-adhesive strength in specific areas of the gastrointestinal tract, such as ileum, small intestine, and colon [27, 28].

Pure psyllium is a safe, suitable polysaccharide for drug delivery system. However, it has various limitations, such as short life, uncontrolled hydration rate, which can be improved through grafting, cross-linking and the complexation with small synthetic molecules [29].

The sustained release properties of psyllium has been explored and evaluated by Desai et al. They formulated the sustained release granules of amoxicilline trihydrate with psyllium matrix [30]. Kaialy et al. have prepared the psyllium and hydroxypropyl

methylcellulose based drug candidate for theophylline drug and achieved an ideal slow drug release profile (80% drug release in 12 h) [31].

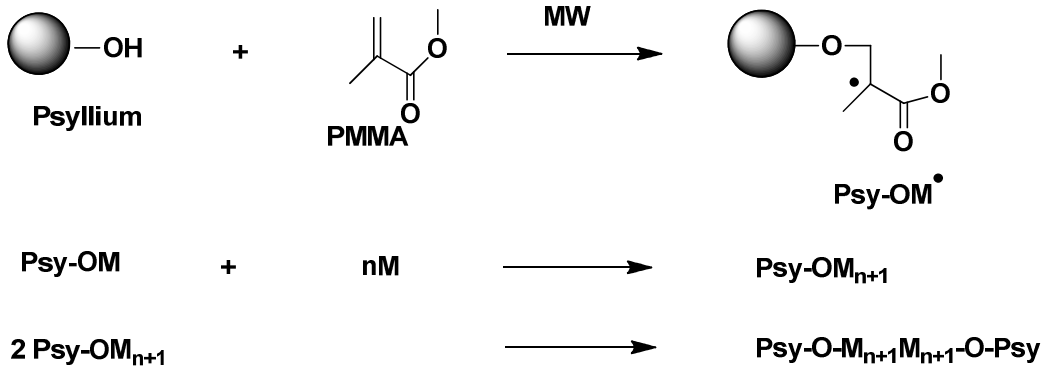
Various researches have suggested psyllium mucilage as the material of high pharmacological importance to decrease the glucose absorption and serve as an excellent drug carriers, if appropriately tailored to synthesize the graft copolymer, therefore, can work as the potential candidates for development of the various types of new drug delivery devices based on graft copolymerization [32].

A large number of synthetic methods of graft copolymerization have been developed. There are three main synthetic methods of graft copolymerization such as (I) conventional chemical method, (II) microwave methods and (III) enzyme method. Among them, the microwave irradiation technique is the best technique for the synthesis of the graft copolymer. It has been exploited in the last two decades to improve the limitations of the synthesis as well as application range of modified polysaccharide materials for various fields and also enhanced the interest in clean and green eco-friendly chemistry. It significantly diminishes the use of toxic solvents, reaction time, increases the product yields, purity, product selectivity and cleans product formations. Microwave synthesized grafted materials show better properties for commercial exploitation than counterparts [33]. After the study by many research groups, it has been found that graft copolymer of natural polysaccharides is an excellent drug carrier and a very good candidate for controlling drug release of therapeutic agents and have extensive applications in controlled drug delivery [34]. The equilibrium swelling capabilities of graft polymers is a balance between elastic forces and swelling of the graft polymers and responds to the variations in environmental factors via their swelling behavior. The swelling behavior of grafted polymers make it useful materials for drug release and use in drug delivery systems [27]. In lieu of this, psyllium and its derivatives can be appropriately designed to improve its swelling and drug release properties. To explain the drug release mechanism from the graft copolymer mathematical modeling plays a significant role. Swelling kinetics of the graft copolymer and drug release kinetics from the graft copolymer depend on the matrix, the composition of the grafted material and pH of solvent and molecular weight of the material, as well as drug, also affects the drug release from the grafted materials. The molecular weight of the drug also affects the release profile of the drug from the grafted polymer [35].

2.2.Earlier method of synthesis

Singh et al, have prepared psyllium-N-vinylpyrrolidone (NVP) based hydrogels through radiation-induced crosslinking. They have studied the swelling kinetics of the hydrogels and drug release [anticancer model drug (5-fluorouracil)] from the hydrogels and found that the release of drug from the hydrogels occurred through Non-Fickian diffusion mechanism [25]. They have synthesized psyllium and methacrylamide based hydrogels in presence of *N, N*-methylenebisacrylamide. The effect of pH on swelling kinetics of the hydrogels and release dynamics of insulin from drug-loaded hydrogels, for the evaluation of the swelling mechanism and drug release mechanism from the hydrogels was also studied. The diffusion mechanism for the release of insulin in pH 2.2 buffers and pH 7.4 buffer The drug released by the polymeric matrix was followed the non-Fickian diffusion mechanism [36]. They have further synthesized the psyllium, polyvinyl alcohol and polyacrylic acid blended hydrogel in presence of ceric ammonium nitrate initiator for drug release study. They chose the antibiotic tetracyclin as a model drug and found that it is released from the psyllium based hydrogel at pH more than 2.2 as compared to pH 7.4. The release of the drug from the hydrogels occurred through non-Fickian diffusion mechanism [37]. Singh et al. have studied the *in vitro* release profile of anti-cancer drug rabeprazole from the psyllium and polyvinyl alcohol-based hydrogel [38]. Singh et al. have also prepared the psyllium and acrylic acid based polymeric networks by using N, N-methylenebisacrylamide (N,N-MBAAm) as crosslinker for removal and separation of hazardous metal ions from aqueous solutions. They also studied the swelling behaviour of grafted material and found that it has the excellent water sorption capacity (700%), which indicates this material is potential candidate for use in colon specific drug delivery. [39].

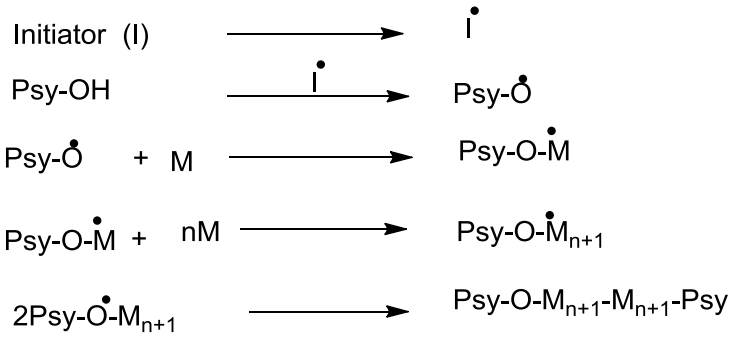
Ganguly et al. synthesized the psyllium-g-poly (acrylic acid-co-sodium acrylate) based nanocomposite for drug delivery. They found that these composites were non-cytotoxic against human cell-line (human osteosarcoma) and shows good cell attachment of live cells in a 5-day 'live dead' assay with almost negligible quantity of cell death [40]. Synthesis and characterization of psyllium seed mucilage grafted with *N, N*-methylene bisacrylamide was also reported by Rao et al. [32]. Mishra et al. studied the microwave synthesis, characterization and applications of polymethylmethacrylate (PMMA) grafted psyllium as a flocculant. The mechanism of synthesis has been depicted in scheme 1 [41].



Scheme 1. Microwave-assisted synthesis of Psy-g-PMMA.

Rosu et al. have prepared the psyllium-based composites containing TiO_2 nanoparticles for aspirin drug delivery. they that it has promising potential for controlled aspirin release as therapeutic agent [42]. Bhatia et al. synthesized the carboxymethylated psyllium arabinoxylan for preparation of polyelectrolyte nanocomposite with chitosan for drug delivery applications [35].

Furthermore so many workers have synthesized various types of grafted psyllium for various applications. Sen et al. reported a novel microwave initiated method for synthesis of polyacrylamide grafted Psyllium (Psy-g-PAM). Psyllium was modified through grafting of polyacrylamide (PAM) chains on it using microwave radiations. The grafting was confirmed by intrinsic viscosity study and characterization techniques like FTIR spectroscopy, elemental analysis (C, H, N, O and S) and SEM morphology study [43]. The mechanism of synthesis of Psy-g-PAM was similar to scheme1. Chaudhary et al. have synthesized the adsorbent based on xanthan gum-psyllium hybrid backbone grafted with polyacrylic acid copolyitaconic acid for effective removal of cationic and anionic dyes. The excellent dye removal efficiency of 90.53% was achieved with 600 mg adsorbent dose within the time duration of 5 h at 50 °C [44]. Kaith et al. have synthesized a novel green psyllium and acrylic acid (AA) based polymeric hydrogel through chemically induced polymerization processes in vacuum. The mechanism of synthesis has been depicted in scheme 2. The study was carried out at the varied time, temperature, pH and NaCl concentration. It has been observed that the hydrogel could absorb the water from the oil-water emulsion to the extent of 4220% [45].



Scheme 2. Schematic representation of mechanism for synthesis of Psy-g-PAA.

Enzymatic modification to improve the water-absorbing and gelling properties of psyllium was reported by Yu et al.[46]. Photo induced grafting of acrylonitrile (AN) onto sodium salt of partially carboxymethylated psyllium (Na-PCMPsy) in presence of ceric ammonium nitrate (CAN) as a photoinitiator in an aqueous medium was studied by Dholakia et al [47].

Basis of work

Psyllium exhibit the wide range of polymeric and medicinal properties and is extensively used as a household medicine since ancient time in all cultures, in various types of diseases, such as cholesterol control, constipation, diarrhoea, duodenal ulcer, to control the high sugar level in blood (diabetes), inflammatory bowel disease colon cancer, piles, weight loss, laxative drug, colon cancer and also used in drug delivery system. Keeping the above facts in the mind, we want to develop the specific drug delivery candidates which have the utility in various type of drug delivery. We were interested to synthesize binary grafted psyllium with acrylic acid and acrylonitrile which is further explored to prepare the formulation with an anti-cancer drug.

2.3.Present work

In the present work, aim to syntheses the binary grafted derivatization of psyllium with acrylic acid as well as acrylonitrile [Psy-g-Poly (AA-co-An)], through the microwave technique without using any initiator. The influence of monomers concentration, microwave power and exposure time, in the reaction mixture on percentage of grafting is investigated and followed by the use of Psy-g-Poly (AA-co-An) based beads for drug release studies of (2-Chloro-3-(4-hydroxyphenylamino) naphthalene-1,4-dione) drug. Anticancer activity of this drug has been already published by Tandon et al. [48]. The synthesized material was characterized by FTIR, SEM, XRD and TGA/DTA/DTG techniques. The drug release

behavior of the beads has been studied at the different pH. The kinetics of drug release were analyzed using the zero, first order and by using Higuchi models.

2.4. Experimental

2.4.1. Materials

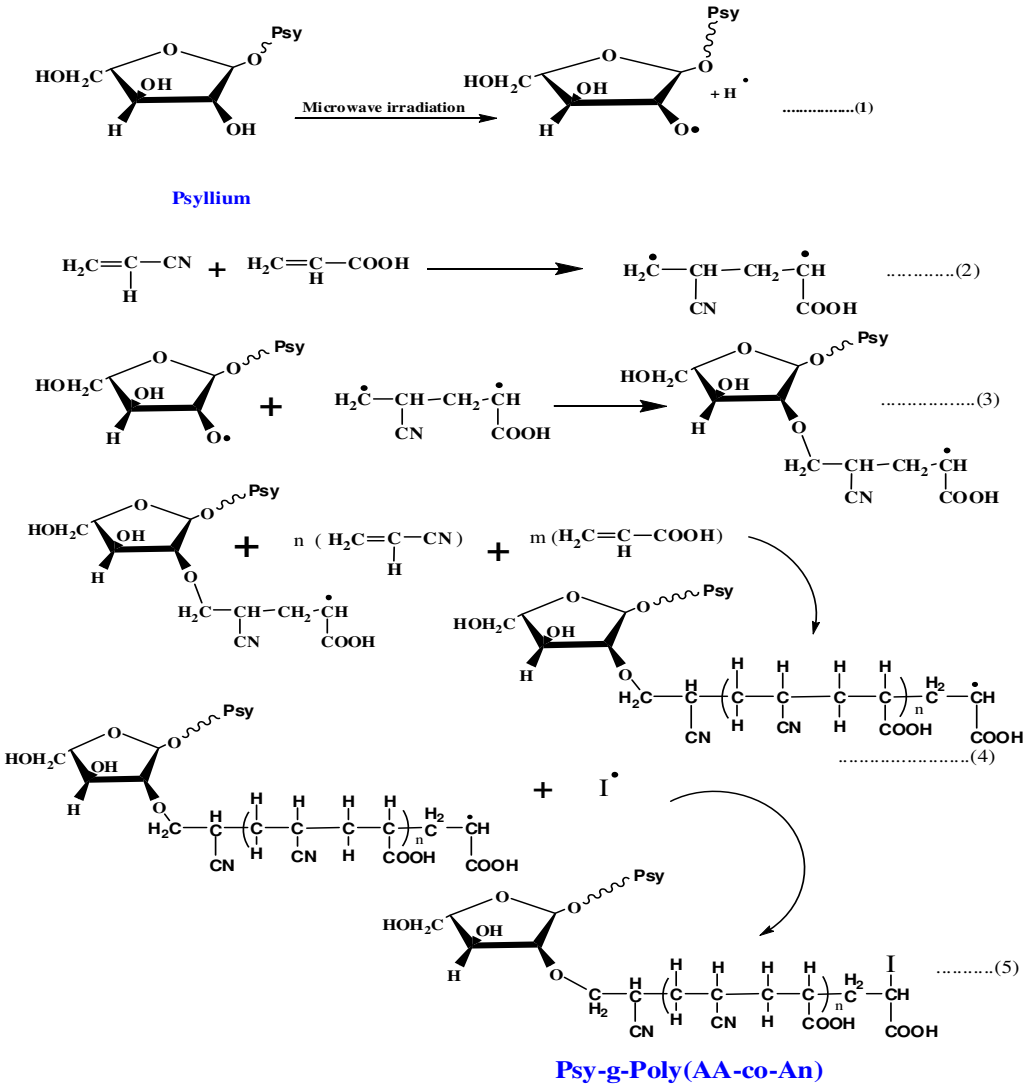
Psyllium husk was procured from Sidhpur Sat-Isabgol Factory India. 50g Psyllium husk was dissolved in 1L distilled water overnight. The mucilage was extracted by filtering through a muslin cloth. Further, the mucilage was precipitated in excess of methanol and filtered through a sintered glass filter and washed with acetone and finally, the precipitate was dried in a vacuum oven at 40 °C. Acrylic acid (AA), acrylonitrile (An), methyl alcohol acetone and hydroquinone were supplied by E. Merck Ltd. Mumbai, India. *N, N*-methylene bisacrylamide (N, N-MBAM) and vitamin C were purchased from Otto Chemie Pvt. Ltd, Mumbai, India and used further without purification. The drug (2-Chloro-3-(4-hydroxyphenylamino) naphthalene-1,4-dione) was obtained as a gift sample by Dr. H. Maurya, Department of Applied Chemistry, Babasaheb Bhimrao Ambedkar University, Lucknow, India. Glutaraldehyde was procured from Central Drug House (P) Ltd New Delhi. Double distilled water was used during all syntheses and application experiments. A domestic microwave (LG model-MH 2548QPS) has been used for grafting of polysaccharide.

2.4.2. Synthesis of Psy-g-Poly (AA-co-An)

1.0 g of psyllium was added to 100 mL distilled water and left undisturbed for an hour to obtain a homogeneous mixture of psyllium in distilled water. After one hour, added the specified amount of acrylic acid (AA) and acrylonitrile (Am) in the solution irradiation under the microwave in a 100 ml conical flask for different time periods and finally reaction was terminated by adding the 0.5 mL of hydroquinone solution. The reaction parameters optimized for maximum grafting at different microwave power and exposure times. The reaction was repeated with 20 mg hydroquinone (radical scavenger) under optimal grafting conditions such as monomers concentration, MW power and exposure time. Psy-g-Poly (AA-co-An) was separated from homopolymer by separating funnel. The graft copolymer [Psy-g-Poly (AA-co-An)] was precipitated in an excess of methanol and the precipitate was washed with acetone and dried in a vacuum oven at 40 °C (Scheme 3).

The % Grafting was calculated by using equation (1)-

$$\% \text{ Grafting } = \frac{\text{wt. of grafted polymer}}{\text{wt. of pure psyllium}} \text{x100} \ldots\ldots\ldots\ldots (1)$$



Scheme 3. Synthesis of Psy-g-Poly (AA-co-An)

2.4.3. Impact of various parameters on grafting

2.4.3.1. Effect of monomer concentration

The effect of concentration of acrylic acid as well as acrylonitrile on to the percentage of grafting was shown in Fig. 17. It was observed that the percentage of grafting was enhanced with increase in the concentration of acrylic acid as well as acrylonitrile from 0.9 to 2.7 mol/L and on further increase in monomer concentration decreases the percentage of grafting due to formation of homopolymer and it was also observed that best grafting occurred at 0.27 mol/L of acrylic acid and 0.7 mol/L of acrylonitrile.

2.4.3.2. Impact of microwave power

The effect of microwave power on to the grafting is shown in Fig 18 a**.** It was observed that the percentage of grafting increases with the increase of the microwave power from 20% to 80% (270 to 1080W) and further increase in the microwave power reduced the percentage of grafting due to formation of homopolymer and thermal degradation of psyllium backbone the highest percentage of grafting (86%) was achieved at 80 microwave power (1080 W).

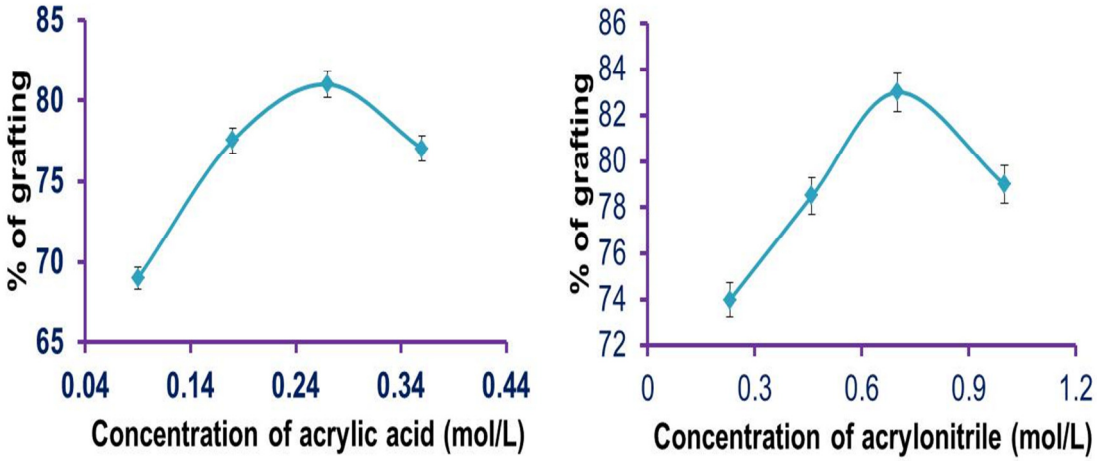


Fig.17. Effect of monomers concentration on grafting.

2.4.3.3. Influence of reaction time onto the grafting

The effect of the reaction time onto the percentage of grafting is shown in Fig. 18 b. The grafting yield was increased with increase in the reaction time. It was observed that the grafting yield increases with increase in time from 30 to 60 seconds and on further increase in time led to slightly increase in grafting.

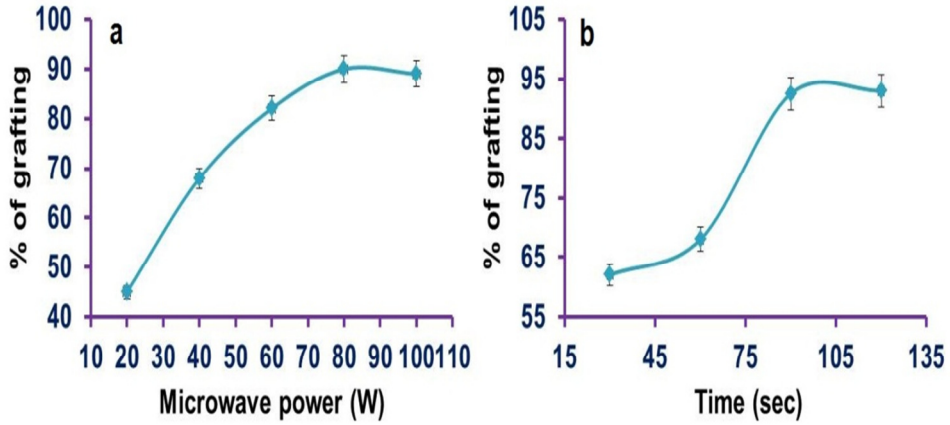


Fig 18. (a) Effect of microwave power on grafting (b) Effect of reaction time onto the grafting.

2.4.4. Characterization of Psy-g-Poly (AA-co-An)

2.4.4.1. FTIR Spectrum

The FTIR spectra of pure psyllium mucilage, binary grafted psyllium and binary grafted psyllium beads are shown in Fig. 19. The FTIR spectra of psyllium mucilage show characteristic peaks at 3472 cm^{-1}, is due to the stretching vibration of O-H and a smaller peak at 2889.37 cm^{-1} due to the C-H stretching vibrations. The band at 1033.85 cm^{-1} is attributed to the C-O-C stretching vibrations, whereas, binary grafted psyllium has some additional peaks as compared to pure psyllium at 1724 carboxylic acid, 1640.52 cm^{-1} (C=O stretching of amide-I) [49], 1360.56 cm^{-1}(N-H in plane bending of amide-II) and 1248.48 cm^{-1} (C-N stretching of amide-III) were observed. The peak position of the drug at 1593 cm^{-1} and 1678 cm^{-1} (> C=O of quinone). In spectra of the binary grafted psyllium beads peaks at 3269 (N-H), and 3468 cm^{-1} (O-H) remain unaltered which indicates that drug does not interact with polymer and its derivatives [50] shown in Fig 19 c. In other words, it can be said that the characteristics of the drug remain as such during the delivery of drug.

2.4.4.2. X-ray diffraction (XRD)

The XRD pattern of pure psyllium mucilage and Psy-g-Poly (AA-co-An) are shown in Fig. 20. In the XRD pattern of pure psyllium mucilage, a broad characteristic peak was observed at 19.3°θ which indicate that pure psyllium mucilage was amorphous nature polysaccharides. The XRD pattern of Psy-g-Poly (AA-co-An) also has a broad characteristic peak at 20.7°θ which also indicated that Psy-g-Poly (AA-co-An) was also amorphous in

nature polysaccharides. The crystallinity of psyllium mucilage was remaining unchanged after the grafting.

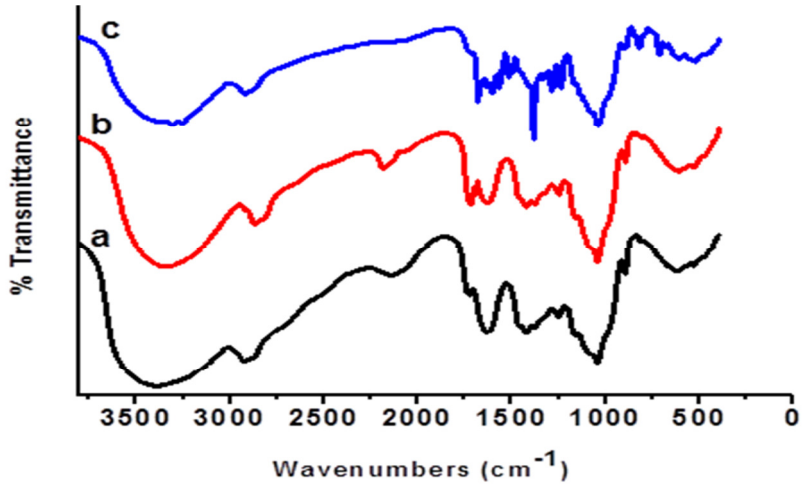


Fig. 19. FTIR spectra (a) pure psyllium (b) Psy-g-Poly (AA-c-An) (c) beads.

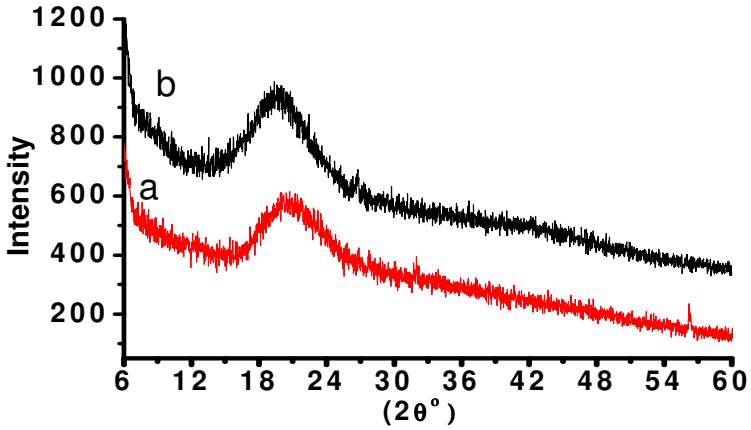


Fig.20. XRD spectra (a) pure psyllium (b) Psy-g-Poly (AA-c-An)

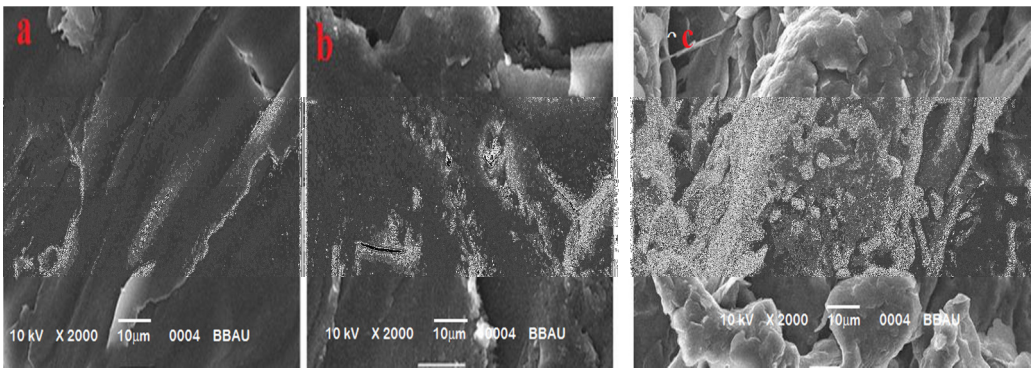


Fig. 21. Scanning electron micrograph of (a) psyllium. (b) Psy-g-Poly (AA-co-An) (c) beads,

2.4.4.3. Scanning electron microscopy (SEM)

The SEM technique is one of the significant techniques to study the surface morphology of various kinds of polymers. A comparative study of the scanning electron micrographs of psyllium mucilage and binary grafted psyllium has also provided a supportive evidence for grafting. The morphology of the surface of psyllium mucilage is totally different as compared to binary grafted psyllium as shown in Fig. 21. It was observed that psyllium has smooth, homogeneous, layered morphology, whereas binary grafted psyllium has heterogeneous and rough surface morphology. The morphology of the surface of the binary grafted psyllium beads was totally rough, heterogeneous and slag like morphology shown in Fig.21c which indicates that the morphology of the surface of grafted gets compound completely changed after the drug loading.

2.4.4.3. Thermal behavior of pure psyllium and Psy-g-Poly (AA-co-An)

Thermal studies (TGA/ DTA/DTG) of pure psyllium and Psy-g-Poly (AA-co-An) are shown in Fig. 22. Thermogravimetric analysis thermograms of pure psyllium and Psy-g-Poly (AA-co-An) were obtained by scanning the sample at the temperature range of 0^{o}C to 800 ^{o}C in an inert atmosphere. From the TGA study of psyllium and Psy-g-poly (AA-co-An), it was found that the decomposition mechanism of both the cases is similar and shows two stages of weight loss and thermograms as shown in Fig. 22 (a and b) respectively. The initial weight loss of pure psyllium mucilage is 12.2 % between the temperature range of 31-250^{o}C which was due to the removal of moisture, and final decomposition (83% weight loss) is noticed up to 442 ^{o}C which was due to degradation of psyllium backbone whereas Psy-g-Poly (AA-co-An) exhibited initial decomposition (13.5% weight loss) at temperature 243°C and final decomposition (96% weight loss) at temperature 500 °C clearly indicates the increase in thermal stability after grafting. Psyllium showed two exothermic peaks at 294 °C (13.4 μV) and 416 °C (98.8 μV). On the other hand, Psy-g-Poly (AA-co-An) exhibited exothermic peaks at 284 °C (13.8 μV) and 413 °C (71.1 μV).

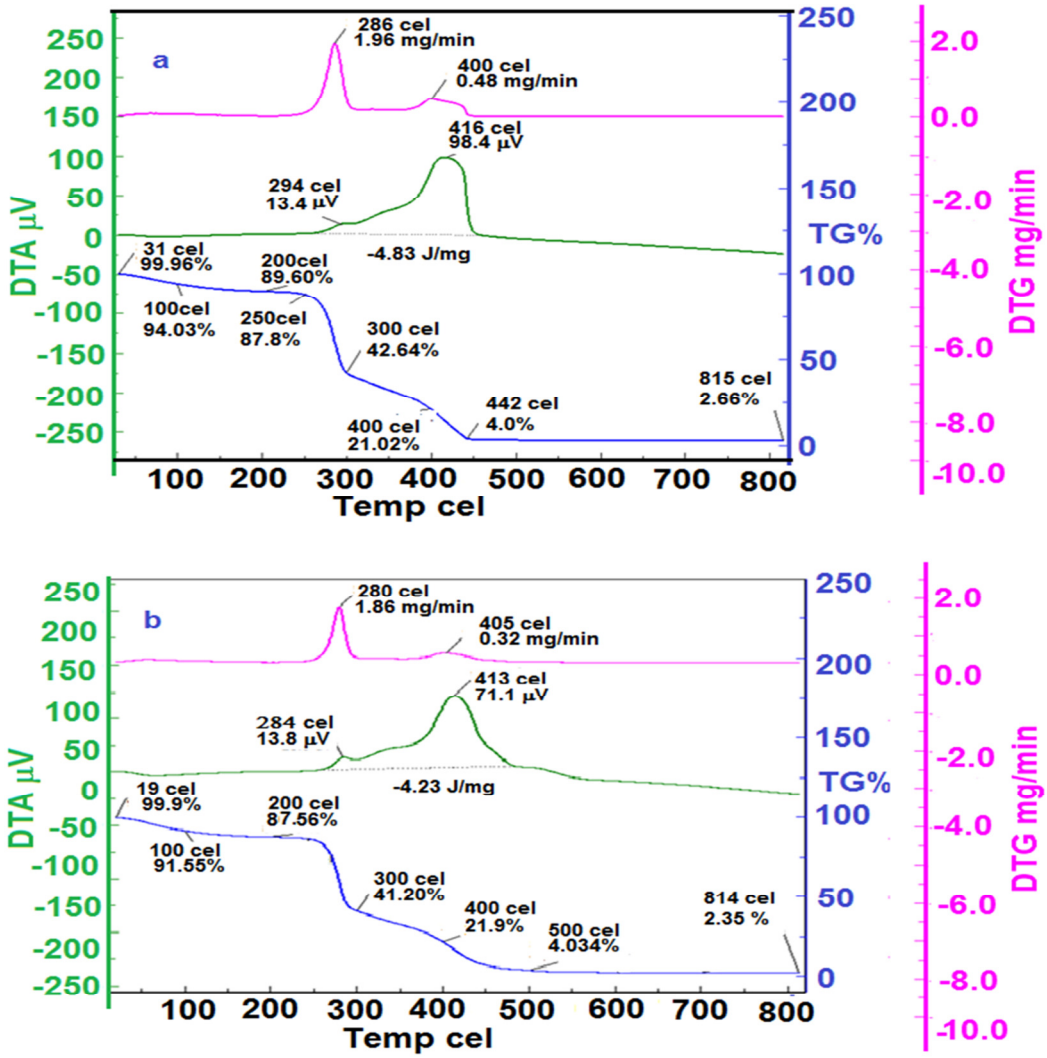


Fig. 22 .TGA, DTA and DTG curves of (a) psyllium (b) Psy-g-poly (AA-co-An).

2.4.5. Measurement of viscosity:

The viscosity is a great significant physicochemical parameter in polymer processing. Viscosity of many polymer solutions was measured by Oswald Viscometer. The method commonly used for determining viscosity coefficient is based on Poiseuille equation (2).

$$\eta \qquad \text{——}\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

Where η = viscosity, V = volume of a liquid, r = radius of capillary tube, L = length of capillary tube, t = time of flow and ρ = pressure.

Absolute viscosity

$$\text{viscosity } (\eta) \text{ sample} = \frac{\text{density of sample x time flow of sample}}{\text{density of water x time flow of}} \text{x viscosity of water} \quad \ldots (3)$$

The variations in viscosity with a concentration of Psyllium and Psy-g-Poly (AA-co-An)] are given in Table-3. It was found that the viscosity increases with increase in the concentration. It was also found that Psy-g-Poly (AA-co-An) has higher molecular weight and viscosity as compared to pure psyllium. The concentration of polymer enhanced the chains of polymer become more entangled and greater shear force is compulsory in order to flow the solution.

Table 3:- Viscosity of Psy-g-Poly (AA-co-An).

S.N	Time of flow for water (Min)	Concentration of sample (g/L)	Time of flow for pure Psyllium(T_{Psy}) (Min)	Time of flow for Psy-g-poly (Min)	Viscosity psyllium (Cps)	Viscosity Psy-g-poly (AA-co-An) (Cps)
1	52	1	56	59	1.0886	1.1469
2	52	2	66	71	1.2856	1.3830
3	52	3	81	87	1.5843	1.7017
4	52	4	91	108	1.9463	2.1232

2.4.6. Swelling behavior

Swelling behavior of Psy-g-Poly (AA-co-An) in water was investigated as the function of time and pH. An accurately weighed amount of the dried Psy-g-Poly (AA-co-An) was placed in a previously dried and weighed glass crucible, which was then filled with 50 mL of solution of different pH, and after different time intervals. The glass crucible was then weighed to determine the amount of water absorbed by per gram of the dried material and

taken as water absorption capacity. Water absorption was measured by the following equation.

$$Water\ absorption\ capacity\ (g/g) = \frac{Weight\ of\ gel}{Weight\ of\ dry\ sample}$$

2.4.6.1. Effect of time on swelling

The Effect of time on swelling of the Psy-g-Poly (AA-co-An) was shown in Fig. 23 a. The swelling behavior of Psy-g-Poly (AA-co-An) was studied at different time intervals of 10 to 180 min. It was found that water absorption capacity of the Psy-g-Poly (AA-co-An) increased with increase in time of swelling.

2.4.6.2. Impact of pH on swelling

The effect of time on swelling of the Psy-g-Poly (AA-co-An) was shown in Fig. 23 b. The swelling behavior of Psy-g-Poly (AA-co-An) was studied at varying pH (pH 2 to 8). It was observed that the water absorption capacity of the Psy-g-Poly (AA-co-An) increased with increase in the pH from 2 to 6 further increases in pH led to decrease in the water absorption capacity of Psy-g-Poly (AA-co-An). The highest water absorption capacity was achieved at 6 pH.

2.4.7. Drug loading/preparation of beads

For the preparation of beads, a known quantity of Psy-g-Poly (AA-co-An) was dissolved in 10 mL distilled water at 25±2^{0}C with continuous stirring to obtain a clear solution. The 0.25 g of 2-chloro-3-(4-hydroxyphenylamino) naphthalene-1, 4-dione drug was added to the resultant solution containing Psy-g-Poly (AA-co-An) and mixed thoroughly. 10 mg of N, N-methylenebisacrylamide (N, N-MBAM) was added to the mixture followed by 20 mL of glutaraldehyde into the reaction mixture with continuous stirring to obtain a homogeneous solution. The reaction mixture was used to prepare the beads into the alkali-methanol solution (1:20 w/w) through the 0.56 mm diameter syringe. The beads were washed with water for removing unreacted glutaraldehyde from beads. The double cross-linked beads were dried at 40 ^{0}C for 24 hours [51, 52]. Drug release based on particulate systems from Psy-g-Poly (AA-co-An) depends upon the extent of grafting, morphology, size and density of the particulate system. *In vitro* drug release from the beads depend upon the polarity and pH of the solution [53] as shown in Fig. 24.

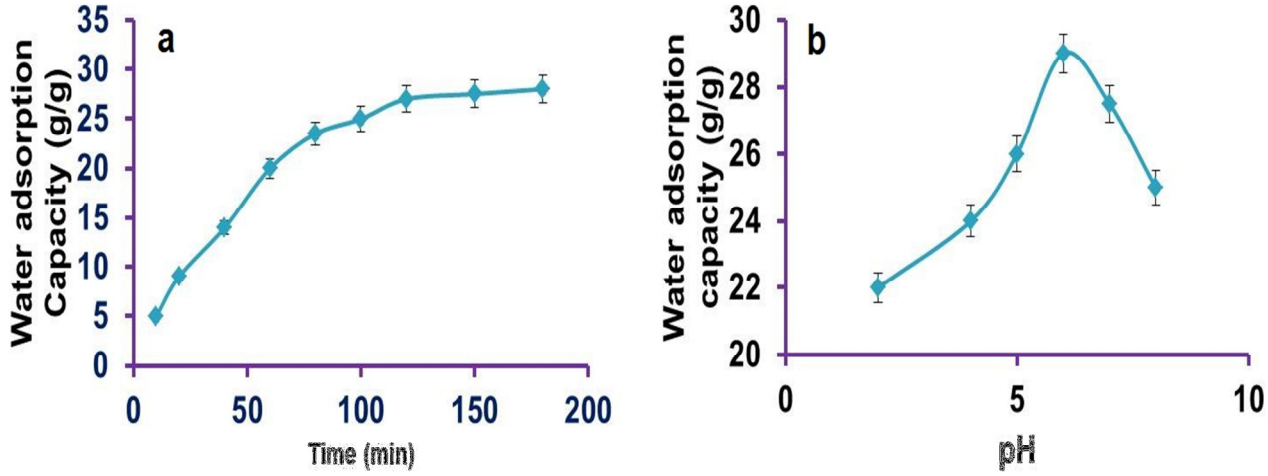


Fig 23. Effect of various parameters on swelling behaviour (a) Effect of pH on swelling (b) Effect of time on swelling.

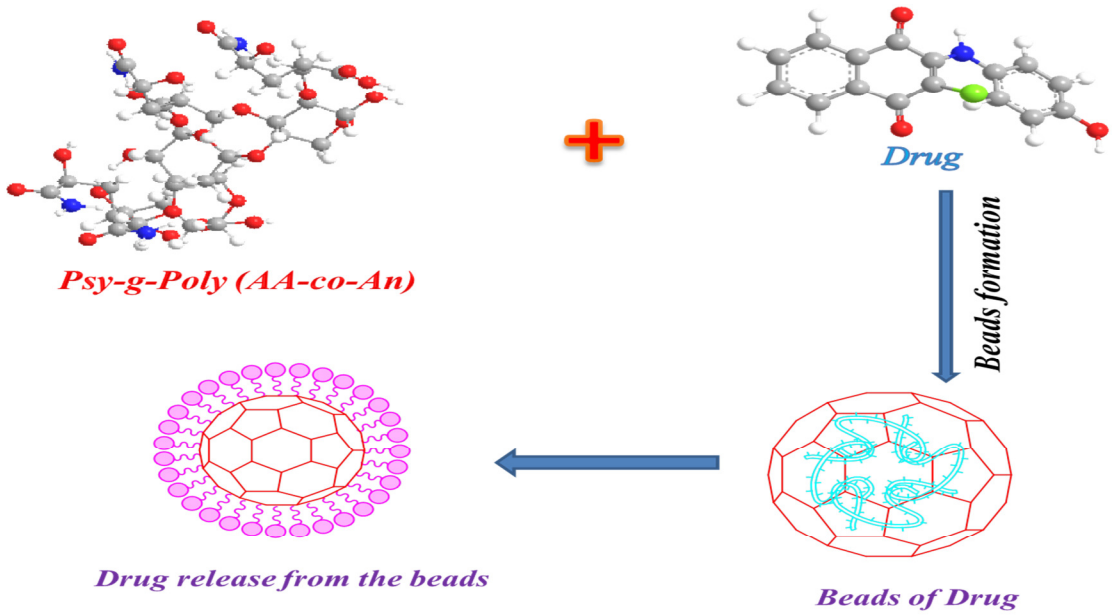


Fig. 24. Schematic representation of drug release from the beads.

The drug release study through the Psy-g-Poly (AA-co-An) based beads was performed in different solutions at 37±1 ºC under unstirred conditions. A known weight of the beads i.e. 20 mg was put in the different solutions at three pH values (1.6, 5.4 and 7.4) [54]. At predefined intervals of time, samples of 5 mL are withdrawn, filtered and assessed

by the absorbance at445 nm through the UV- spectrophotometer (systronics 2203). Beer Lambert's equation (5) is used for the drug release study.

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2.4.7.1. Influence of time on drug release

Impact of time on drug release at various pH is shown in Fig. 25, where it was observed that drug release from the beads increases by enhancing the time.

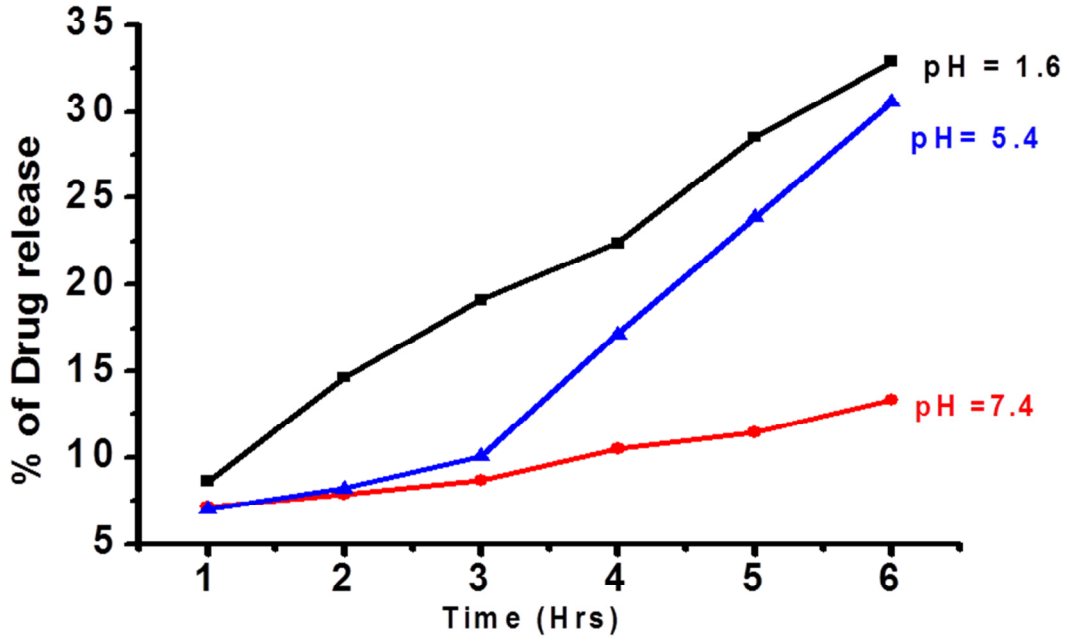

Fig. 25. Impact of time on drug release at various pH.

2.4.8. Kinetic analysis of drug release

The *in vitro* kinetics of drug release from the beads in the dissolution media was studied and analysed with several types of kinetic models like zero-order, first order and Higuchi's model [55, 56].

2.4.8.1. Zero-order reaction

Zero-order kinetics was studied to explain the drug solubility of the drug from many types of improved release [11]. This model is important in different types of medicines e.g. for heart and blood pressure maintenance, antibiotic delivery, pain control and antidepressants [57].

The linear form is given as equation (6).

$$\frac{Q}{Qo} = Kot \ldots\ldots\ldots (6)$$

Q = Amount of drug release, Q_0 = Original amount of drug, K_0= Zero order rate constant, t = time

The regression R^2 obtained by the linear plot of % of drug released verses time, (Fig 26 a), is shown in table-4.

2.4.8.2. Higuchi

A Higuchi's model, an inactive matrix should display a continual drug release for a rational time period as well as percent drug release and obtain the straight line when the plotted graph drug released *vs* $t^{1/2}$ shown in Fig. 26 b [58]. In order to have an insight into the mechanism of drug release behavior, the diffusion arrangement of the drug is noticed by solution penetration rate [59, 60]. The drug release mechanism is shown by the kinetic equation (8)

$$\frac{Mt}{M\infty} = Kt^{\frac{1}{2}} \ldots\ldots\ldots\ldots\ldots (8)$$

Where Mt/M∞, is the fractional release of the drug at time t, K is the constant related to the structural and geometric characteristic of the device and the regression R^2 value given in Table-4.

2.4.8.3. First order reaction

First time Feldman and Gibaldi (1967) studied the first order drug release kinetics to explain the drug solubility in Pharmaceutical dosage forms in polymer [61]. The first order kinetics define the drug release from the bead/formulation with respect to time and concentration by the following equation 7 [59].

$$\ln\frac{Qo}{Qt} = k_1 t \ldots\ldots\ldots\ldots (7)$$

Q_o and Q_t is a concentration of drug at t=0 and t=t time respectively. K_1 is the rate constant. The regression R^2 obtained by the linear plot of ln (Qo/Qt) Vs t (Fig 26 c), is shown in Table-4. For drug release, R^2 was greater than 0.9, which designates a good fit to the investigational data [62].

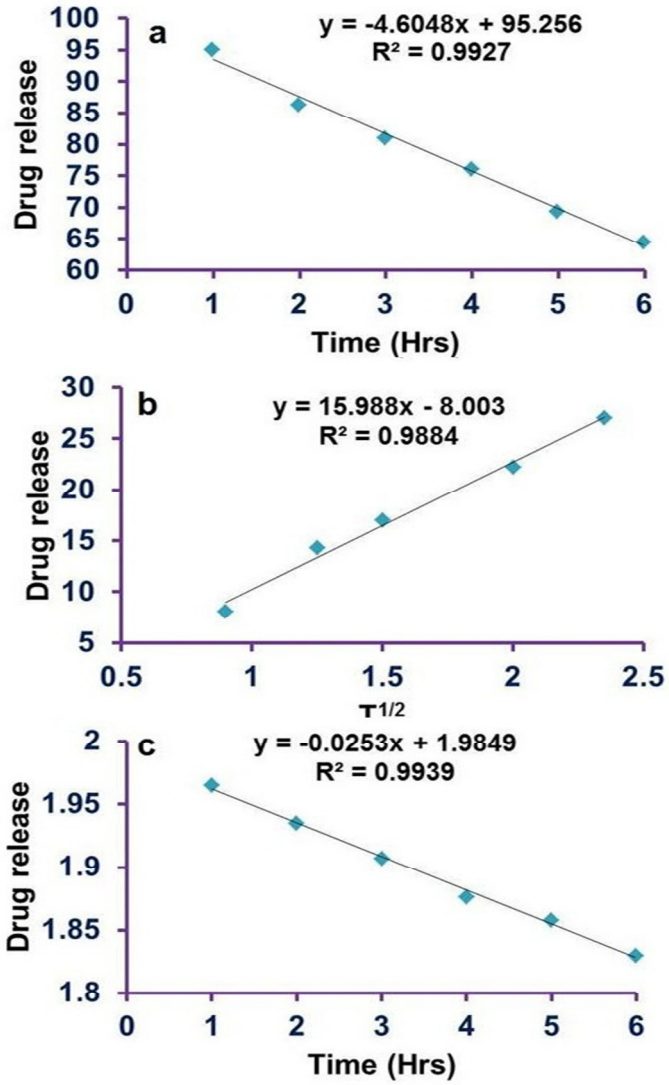

Fig. 26. Kinetic study of drug release (a) Zero-order reaction drug release kinetics (b) Higuchi model of drug release kinetics (c) First order reaction drug release kinetics

Table 4:- *In vitro* drug release kinetics models fitting.

S.N.	Drug release kinetics models	k	R^2
1	Zero-order	4.6	0.9927
2	First order	0.057	0.9939
3	Higuchi	11.16	0.9884

2.5. Conclusion

A binary grafted derivative of Psyllium with acrylic acid and acrylonitrile (Psy-g-Poly (AA-co-An)) was synthesized successfully through the microwave technique. The remarkable difference in extent of grafting was observed with the variation in the concentrations of acrylic acid and acrylonitrile, microwave power and reaction time. The grafting of acrylic acid and acrylonitrile onto psyllium mucilage chain was confirmed by FTIR, XRD, SEM, and TGA/DTA/DTG analytical techniques. Grafting of acrylic acid as well as acrylonitrile on psyllium mucilage, offer noble polymeric materials with characteristics that can be exploited industrially. It was also observed that Psy-g-Poly (AA-co-An) has the excellent water absorption capacity which depends on time and pH variation. It was also found that Psy-g-Poly (AA-co-An) is pH sensitive polymeric material. We have successfully prepared the Psy-g-Poly (AA-co-An) based, pH-sensitive beads for drug release. The result demonstrated that the drug release depends on the pH of the solution; it was found that maximum *in vitro* drug release at 1.6 pH > 80 % in 24 hours. On fitting the in vitro release data of beads to different types of kinetic models, it was observed that it shows Higuchi order of kinetics followed by zero order and first order. The drug release kinetics of the beads shows anomalous (non-Fickian) diffusion. However, the polymeric properties of Psy-g-Poly (AA-co-An) are likely the milestone in the area of pharmaceuticals with respect to the development of pharmaceutical dosage.

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Chapter -3

Synthesis and characterization of modified chitosan via microwave route for antibacterial application

We report herein the synthesis of novel antibacterial graft [Chit-g-Poly (AA-co-An)] and crosslink [Chit-cl-Poly (AA-co-An)] copolymer, consisting of acrylic acid (AA), acrylonitrile (An) and chitosan by using the microwave route, where it has been found that grafting and crosslinking copolymers have excellent antimicrobial properties. Studies of antibacterial activities of graft and crosslink samples were carried out against gram positive [*Staphylococcus aureus (S. aureus*)], gram negative [*Escherichia coli (E. coli)* and *Pseudomonas aeroginosa (P. aeroginosa*)] bacteria. The graft [Chit-g-Poly (AA-co-An)] and crosslink [Chit-cl-Poly (AA-co-An)] copolymers were characterized by Fourier transform infrared spectroscopy (FTIR), scanning electron micrography (SEM), thermogravimetric analysis (TGA), X-ray diffraction (XRD). techniques to study structural characteristics of synthesized chitosan derivatives. The graft [Chit-g-Poly (AA-co-A)] copolymer shows excellent antibacterial activities against *E. coli*, *P. aeroginosa* and *S. aureus* 30, 31 and 26 mm zone inhibition, respectively meanwhile [Chit-cl-Poly (AA-co-A)] shows antibacterial activities against *E. coli*, *P. aeroginosa* and *S-aureus* 26, 36 and 21 mm zone inhibition respectively.

Chapter-3 *Synthesis and characterization of modified chitosan via microwave route for antibacterial application*

3.1. Introduction

Chitosan (-(1,4)-2-amino-2-deoxy-d-glucose, is the partially and fully *N*-deacetylated derivative of chitin, and this *N*-deacetylation of chitin is always go on and never complete [1]. Apart from this, chitosan is a renewable resource and have unique properties such as biodegradable, biocompatibility, non-antigenicity, non-toxicity and antibacterial activity [2]. Due to such unique properties, chitosan has the potential applications in various fields such as agriculture, biomedicine, environmental protection, waste-water treatment, cosmetics, functional membranes and the food industry [3].

Moreover, the cationic characteristic of chitosan permits it to show superior inhibitory activity against an inclusive variety of microorganisms [4], including trypanosomes [5], fungi and bacteria [6]. Positively-charged chitosan have tendency to interact with negatively-charged microbial cell membranes leading to alterations in cell wall permeability and the leakage of intracellular compounds [7]. However, factors including degree of deacetylation, molecular weight [8] and positive charge content can disturb the antibacterial activities of chitosan [9]. Modified chitosan with sulfonate groups or quaternary ammonium groups or vinyl monomers, have better antimicrobial activities as compared to pure chitosan [10].Chitosan and its derivatives have attracted much attention as antimicrobial agents against bacteria, fungi and viruses [11]. Chitosan has many advantages over other antimicrobials due to its high, broad-spectrum antimicrobial activity and decreased toxicity toward mammalian cells [12]. There exist a number of commercial applications of chitosan that take advantage of this antimicrobial activity, such as in food preservation and packaging [13]. Chitosan has been assessed for several uses in the food, medical, pharmaceutical, agricultural and chemical industries because of it's nontoxic, biocompatible, mucoadhesive and biodegradable characteristics [14, 15] and also improve food quality and extend shelf life by minimizing microbial growth in the product. Therefore, Korea and Japan have approved the chitosan as a food additive in 1983 and 1995 respectively.[16-18].

Graft/cross-link copolymerization is a very common and significant technique to modify the chitosan. Enzymatic and chemical modifications of chitosan were done due to presence of free reactive amino and hydroxyl groups. The enzymatic modifications are costlier than other methods whereas the reagent used for chemical modification is harmful to the environment; therefore, other amended processes have also been investigated [3, 19]. Irradiation modification of chitosan is unique, clean, green and environmentally friendly method among all the known techniques to introduce new properties with minimum loss of the initial properties and increase its potential utility in various applications [20, 21]. The grafting of pure chitosan enhances the antimicrobial activities of chitosan either through complexing with essential transitional metal ions which makes these ions unavailable for bacteria or binds to the negative charged bacterial surface to disturb the cell membrane. Therefore these grafted chitosan can be used in wound-healing management for e.g. carboxymethyl-chitosan is used to reduce the periodontal pockets in dentistry and chitosan grafted with EDTA is used as a constituent of hydro- and hydroalcoholic gels for topical use, because chitosan and its derivatives exhibit the bacteriostatic activity [22]. Valenta et al. also observed that grafted chitosan (carboxymethyl-chitosan) has the excellent antimicrobial activity as compare to pure chitosan which makes essential transition metal ions unavailable for bacteria or binds to the negatively charged bacterial surface to disturb the cell membrane [23].

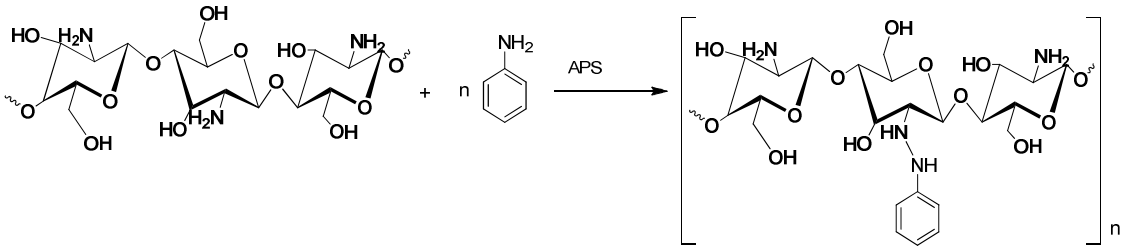
3.2. Earlier method of synthesis

In 2015, Khalil et al. have synthesized the water-soluble derivative of chitosan via grafting of dicyandiamide (DCDA) onto chitosan. The DCDA-grafted chitosan has shown antibacterial activity against *E. coli* and *S. aureus* at pH of 7 and 4 respectively [24]. They proposed that $-NH_2$ and cyano groups of DCDA in grafted chitosan interact with the bacterial membrane and causing disruption. Klaykruayat et al. reported that grafting of cationic hyperbranched dendritic polyamidoamine with terminal methyl ester on chitosan resulted in increase in water solubility of grafted derivative and exhibited antimicrobial activity against *S. aureus* [25].

Five chitosan derivatives namely chitosan O-(benzene) triazolyl carbamate, (chitosan *O*-(adamantane) triazolyl carbamate, chitosan *O*-(1-methylbenzene) triazolyl carbamate, chitosan *O*-((R)(1-methyl)-1-Bocpyrrolidine) triazolyl carbmate) and chitosan *O*-(1-methyl phenyl sulfide) triazolyl carbamate have been synthesized and biologically screened against three gram-positive and gram-negative bacteria and three fungal strains by Sarwar et al. they found that MIC value of all derivatives ranged between 188–1500 g/mL for fungi and 31.3 to 250 g/mL for bacteria [26].

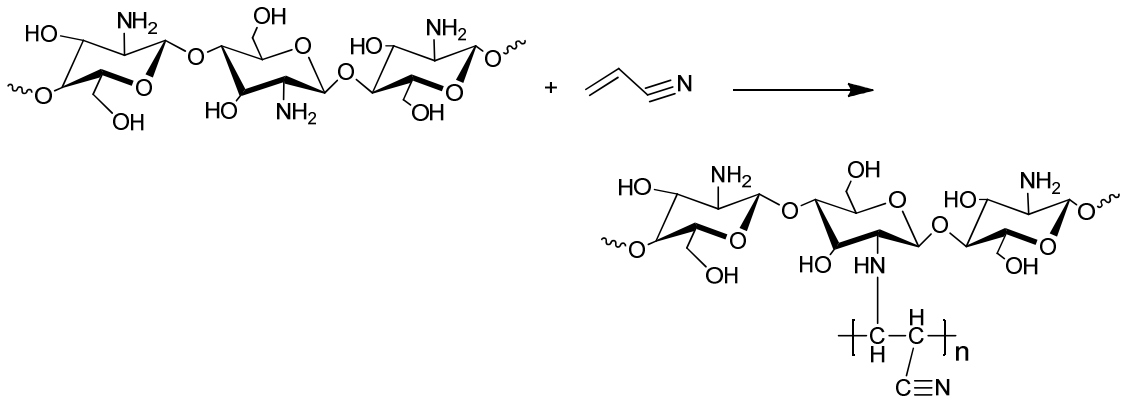
Kohsari at el. synthesized the antimicrobial chitosan-polyethylene oxide and also evaluated the antimicrobial activities of chitosan-polyethylene oxide against *Staphylococcus aureus*and *Escherichia coli* bacteria through viable cell-counting method [27]. Yang et al. investigated the antibacterial activities of cinnamic acids grafted chitosan against *R. solanacearum,* especially RS-5, which is the pathogenic bacterium of mulberry bacterial wilt and plant pathogenic bacteria [28].

Furthermore so many workers have synthesized various type of grafted chitosan for various applications. Khairkar et al. synthesized the chitosan-graft-polyaniline (Ch-g-PANI) in the presence of ammonium persulfate in acidic medium by a conventional method. The utility of Ch-g-PANI was in the electronic devices especially for the fabrication of sensor devices [29]. The mechanism of the synthesis of Ch-g-PANI was given in scheme 1.



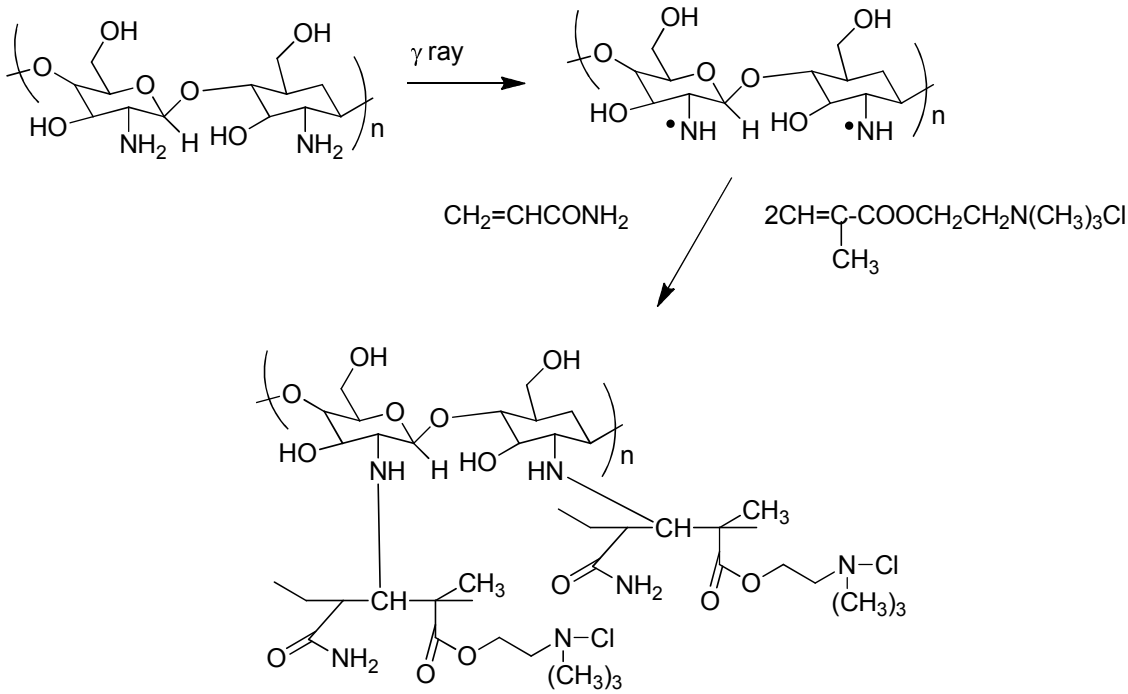
Scheme 1. Schematic representation of mechanism of the synthesis of Ch-g-PANI.

Aguilar et al. synthesized the polyacrylonitrile-g-chitosan (PAN-g-CS) in the presence of an initiator ceric ammonium nitrate (CAN) via conventional method for the removal of Pb^{2+}, Cd^{2+} and Zn^{2+} ions in aqueous solutions. The mechanism of synthesis of PAN-g-CS has been shown in scheme 2 [30].



Scheme 2. Schematic representation of mechanism of the synthesis of PAN-g-CS.

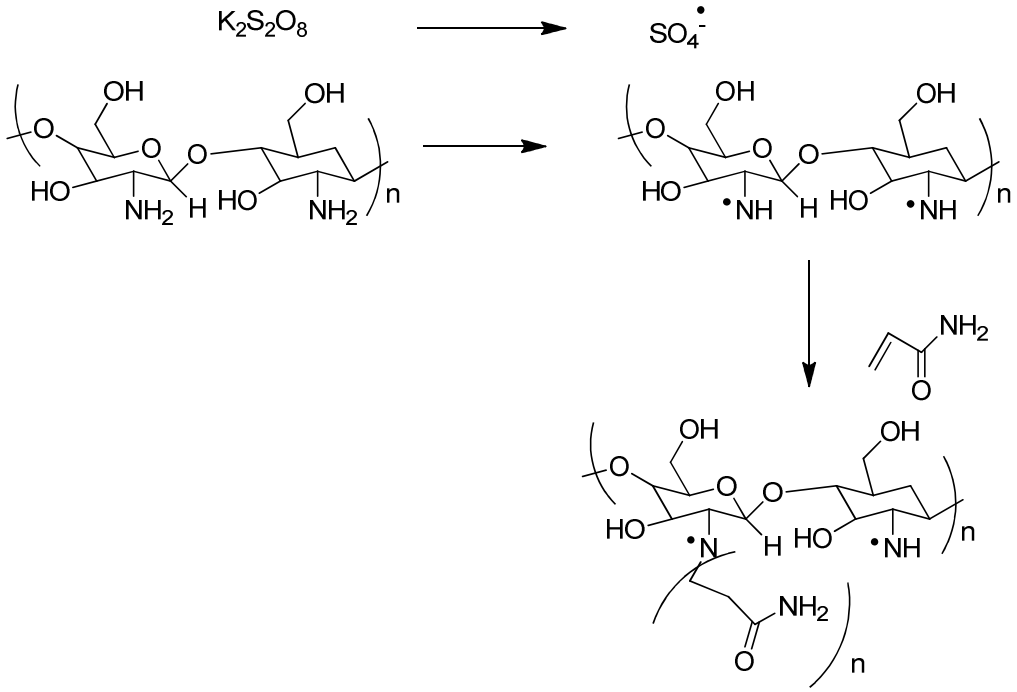
Wang et al. synthesized the binary grafted chitosan with two monomers [acrylamide and (2-methacryloyloxyethyl) trimethyl ammonium chloride] via γ - radiation at ambient temperatures and used it as a cationic flocculant. The reaction process is illustrated in Scheme 3 [31].



Scheme 3. Schematic representation of graft-copolymerization of chitosan.

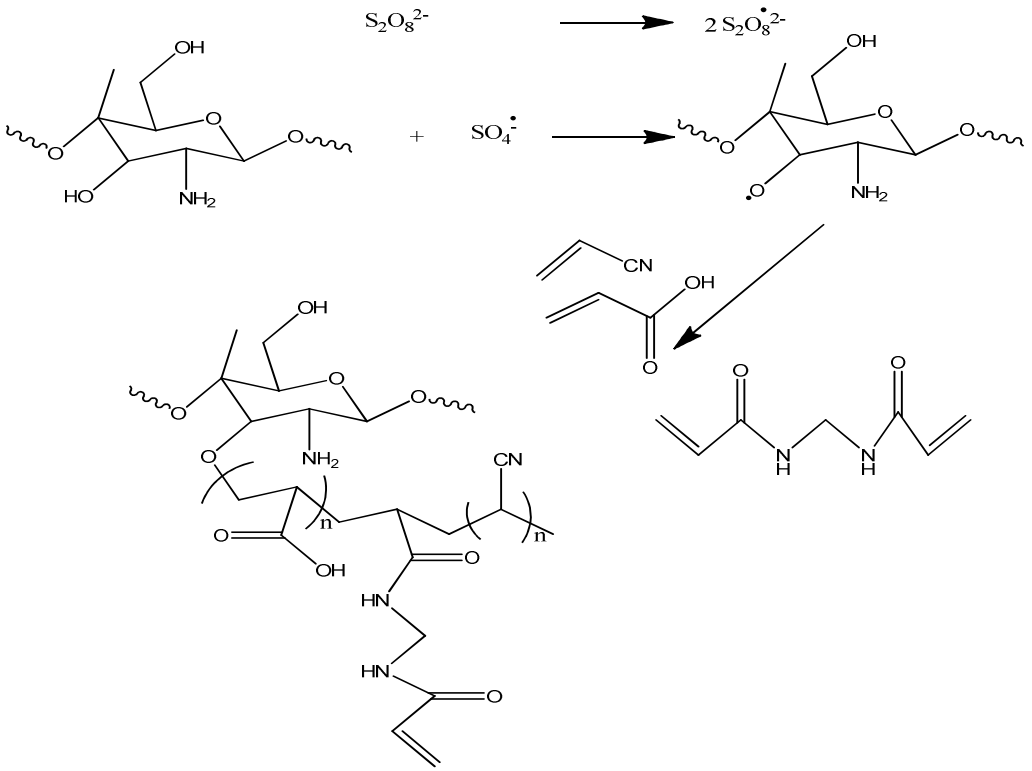
Soliman et al. synthesized the chitosan based flocculants by conventional peroxy graft copolymerization of acrylamide onto carboxymethyl chitosan in presence

of potassium persulphate (PPS) initiator The reaction process is illustrated in Scheme 4 [32].



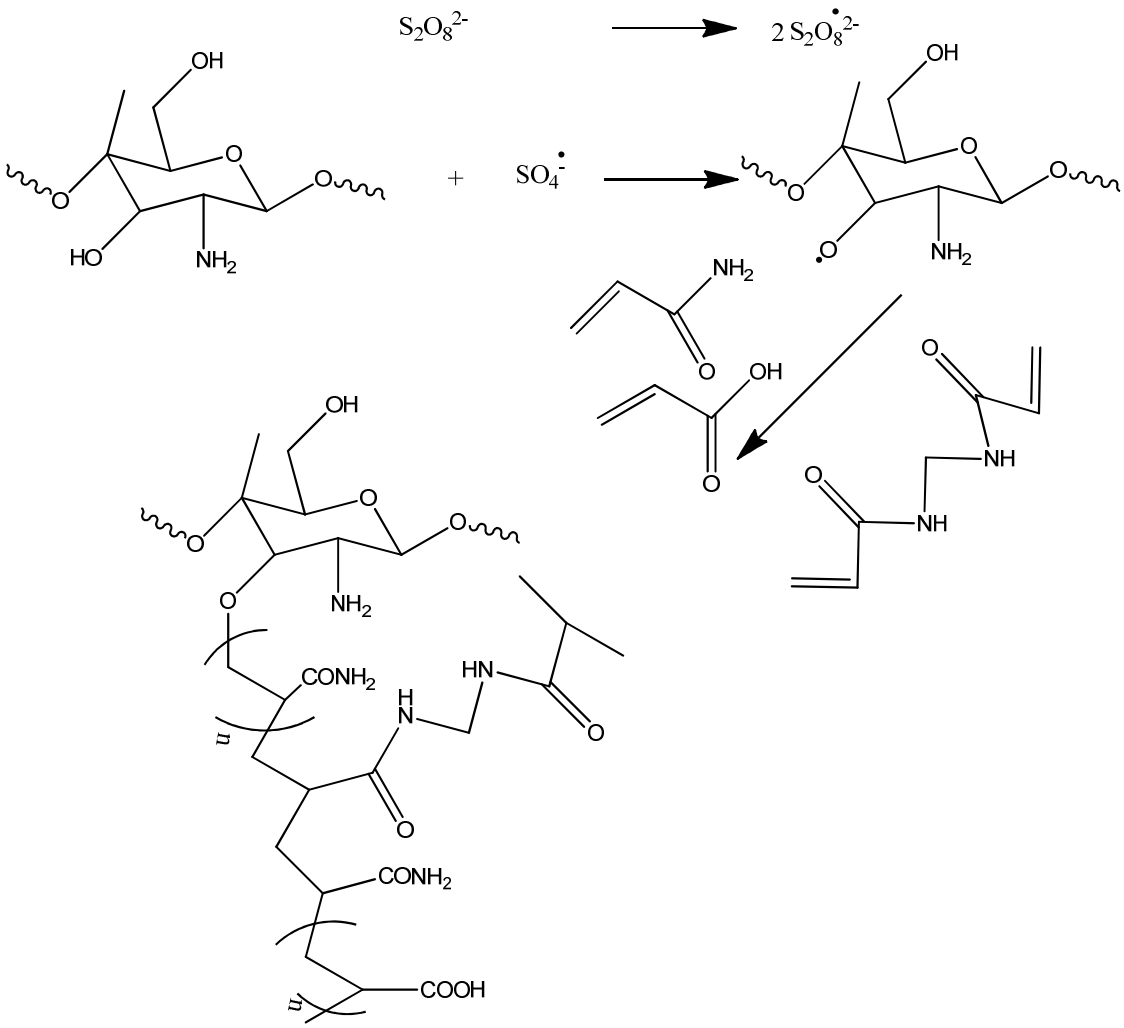
Scheme 4. Schematic representation of grafted chitosan.

Sadeghi et al. have synthesized the super absorbent hydrogel based on chitosan-g-poly (acrylic acid-co-acrylonitrile) in presence of ammonium persulfate (APS) initiator and methylenebisacrylamide (MBA) cross-linking agent under an inert atmosphere. The mechanism of synthesis of chitosan-g-poly(acrylic acid-co-acrylonitrile) has been shown in scheme 5 [33].



Scheme 5. Schematic representation of chitosan-g-poly (acrylic acid-co-acrylonitrile).

Mahdavinia et al. synthesized the super absorbent hydrogel based on chitosan-g-poly (acrylic acid-co-acrylamide) in presence of ammonium persulfate (APS) initiator and methylenebisacrylamide (MBA) cross-linking agent under an inert atmosphere. The mechanism of synthesis of chitosan-g-poly (acrylic acid-co-acrylamide) has been shown in scheme 6 [34].



Scheme 6. Schematic representation of chitosan-g-poly (acrylic acid-co-acrylamide).

3.3. Basis of work

Chitosan exhibit the wide range of polymeric and medicinal properties. Chitosan is a biological active natural polysaccharide and possesses very interesting biological properties, namely cytocompatibility, antimicrobial, antioxidant, anti-cholesterolemic, anti-inflammatory, analgestic, haemostatic, mucoadhesion. Therefore, chitosan has been used in a variety of applications, most relevant in the medical as well as pharmaceutical fields. All the above fact helps us to design and develop the binary grafted and cross-linked chitosan for antibacterial application.

3.4.Present work

In the present study, we report the synthesis and characterization of novel antibacterial, grafted and crosslinked copolymers of chitosan with acrylic acid and acrylonitrile under microwave irradiation. The *in-vitro* antimicrobial activity of the graft [Chit-g-Poly (AA-co-An)] and crosslink [Chit-cl-Poly (AA-co-An)] copolymers were evaluated using agar culture, including waterborne food pathogens *Escherichia coli (E. coli)and Staphylococcus aureus (S. aureus)* along with air borne pathogen *Pseudomonas aeruginosa* (*P. aeruginosa*) and experiments were carried out using the optimum sample.

3.5.Experiments

3.5.1. Materials

All the experiments were carried out on a domestic microwave oven (LG. MH 2548QPS). Chitosan (viscosity < 200 cps, molecular weight ~ 40 kDa, deacetylation 80%) was purchased from Jaipur Scientifics and Chemicals Bareilly, India (Otto Chemie Pvt Ltd, Mumbai, India). Acrylic acid (AA) and acrylamide (Am) were supplied by Jaipur Scientifics and chemicals Bareilly, India (E. Merck, India). Acetone, methanol, Ceric Ammonium Nitrate (CAN) and hydroquinone were purchased from Jaipur Scientifics and chemicals Bareilly, India (Rankem, New Delhi, India). *N,N*-methylenebis acrylamide (*N,N* MBAM) and vitamin C were purchased from Jaipur Scientifics and chemicals Bareilly, India (Otto Chemie Pvt Ltd, Mumbai India). All the chemicals were used such as received without any further purification.

The percentage of grafting and crosslinking ware calculated by the following equations-

$$\text{Grafting (in \%)} = \frac{\text{wt. of grafting polymer}}{\text{wt. of pue chitosan}} \text{x100}$$

$$\text{Crosslinking (in \%)} = \frac{\text{wt. of crosslinking polymer}}{\text{wt. of pure chitosan}} \text{x100}$$

3.5.2. Synthesis of the Chit-g-Poly (AA-co-An) and Chit-cl-Poly (AA-co-An)

The graft and crosslink copolymers of chitosan with acrylic acid (AA) as well as acrylonitrile (An) was synthesized through graft and crosslink co-polymerization. 1.0 g

chitosan was dissolved in 100 mL 2% of the acetic acid solution and left undisturbed for one hour to obtain a homogeneous mixture of Chitosan in 2% of the acetic acid solution. After one hour, added the specific amount of acrylic acid (AA) and acrylamide (Am) in the solution under the microwave irradiation in a 100 mL conical flask for a series of microwave powers and then terminated by injecting 0.5 mL of saturated aqueous hydroquinone solution [21, 35]. The reaction product was precipitated out in excess of methanol and the precipitate was washed with acetone and finally, the precipitate was dried in a vacuum oven at 40 °C. For crosslinking the required amount of *N,N*-methylenebis acrylamide were added to the reaction mixture after the monomers.

3.5.3. Influence of reaction parameters on grafting/crosslinking

3.5.3.1. Effect of concentrations of Acrylic acid, acrylamide and N, N-methylene bis acrylamide

The effect of Acrylic acid, acrylamide and *N,N*-methylenebisacrylamide concentration on percent grafting/cross-linking is shown in Table-5, with increase in the concentration acrylic acid and acrylamide from 0.010 to 0.030mol/L and 0.4 to .08 mol/L respectively the percentage of grafting increases but on further increase of monomer concentration results indecrease in grafting percentage. The increase of percentage of grafting was expected with increase in acrylic acid as well as acrylonitrile concentration due to the availability of acrylic acid as well as acrylamide monomers with respect to polysaccharide macroradicals, leading to larger possibility of grafting, but the decrease in grafting percentage after addition of 0.04 mol of AA and 1.1 mol An might be due to the formation of more homopolymer. The percentage of cross-linking increased from 71.62% to 89.11% with increase in the concentration of *N, N*-methylene bis acrylonitrile 0.12 $x10^{-3}$ to $0.36x10^{-3}$mol/L and further increase in concentration results in the decrease of crosslinking percentage.

3.4.3.2. Effect of microwave power

The effect of microwave power on the grafting is shown in Table-5. It was investigated that the grafting is increased with the increase in the microwave power from 40% to 80% and further increase in the microwave power reduced the grafting/

cross-linking yield. The best percentage (89.3%) grafting was observed at 80% (1080 W) microwave power.

3.4.3.3. Effect of reaction time onto the grafting

The effect of the reaction time onto the grafting/cross-linking yield is given in table-5. The grafting yield was increased with increase in reaction time. It was observed that the grafting yield increases with increase in time from 30 to 90 seconds and further increase in time from leads to slight increase in grafting.

Table 5:- Effect of concentrations of monomers, cross-linker, microwave power and time on percentage grafting/cross-linking.

S.N.	Acrylic acid (mol/L)	Acrylonitrile (mol/L)	N, N MBAM (mol)x10^{-3}	Microwave	Time	Grafting (%)	Crosslinking (%)
1	0.010	0.5	-	60	120	72	-
2	0.02	0.5	-	60	120	76	-
3	0.03	0.5	-	60	120	80.5	-
4	0.04	0.5	-	60	120	77	-
5	0.03	0.4	-	60	120	73.6	-
6	0.03	0.8	-	60	120	84.4	-
7	0.03	1.1	-	60	120	81.2	-
8	0.03	0.8	-	40	120	75.2	-
9	0.03	0.8	-	60	120	84.5	-
10	0.03	0.8	-	80	120	89.26	-
11	0.03	0.8	-	100	120	87.3	-
12	0.03	0.8	-	80	30	54.6	-
13	0.03	0.8	-	80	60	78	-
14	0.03	0.8	-	80	90	85.3	-
15	0.03	0.8	-	80	120	89.5	-
16	0.03	0.8	-	80	150	91.7	-
17	0.03	0.8	0.010	80	120	-	71
18	0.03	0.8	0.020	80	120	-	78.2

19	0.03	0.8	0.03	80	120	-	83.6
20	0.03	0.8	0.04	80	120	-	89.4
21	0.03	0.8	0.05	80	120	-	87.1
22	0.03	0.8	0.06	80	120	-	84.2

3.4.4. Characterization of Chitosan, Chit-g-Poly (AA-co-An) and Chit-cl-Poly (AA-co-An)

3.4.4.1.FT-IR analysis

The FTIR spectra of chitosan and chit-g-Poly (AA-co-An) and chit-cl-Poly (AA-co-An) are shown in Fig. 27. A broad peak has been observed around 3420 cm^{-1} in pure chitosan, due to the stretching vibration of O-H and the vibration of N-H. Peaks at 1420 cm^{-1} and 1377 cm^{-1} are due to N-H stretching of the amide and ether bonds respectively. In the cross-linked sample, the reduction in the intensity of the peak (at 3431 cm^{-1}) was recorded due to the overlapping of O-H stretching and N-H stretching of amide groups in chitosan. It is clear that the cross-linked copolymer chit-cl-Poly (AA-co-An) has two additional peaks at 2248 cm^{-1} and 1724 cm^{-1} which are the characteristic peaks of CN group of acrylonitrile and COOH group of acrylic acid, which is evident of cross-linking in chitosan as shown in Fig.27 b.The infrared IR spectrum of the chit-g-Poly (AA-co-An) was shown in Fig. 27 c. In the grafted sample, the reduction in the intensity of the peak (at 3401 cm^{-1}) was recorded due to overlapping of O-H stretching of chitosan and N-H stretching of amide groups. The two absorption bands were found at 2235 cm^{-1} and 1722 cm^{-1} which indicate the grafting of acrylonitrile and acrylic acid on to chitosan. The other peak at 2873$cm^{-1}$was arise due to C-H stretching, which diminished in grafted and cross-linked samples i.e. free C-H stretching hindered after modification of chitosan [36].

3.4.4.2.SEM and EDS Study

The surface of the chitosan, chit-g-Poly (AA-co-An) and chit-cl-Poly (AA-co-An) was examined by scanning electron microscopy. The scanning electron micrographs of chitosan, chit-g-Poly (AA-co-An) and chit-cl-Poly (AA-co-An) are shown in Fig. 28. The surface of pure chitosan was rough, heterogeneous and porous.

Grafted sample [chit-g-Poly (AA-co-An)] had the smooth, homogeneous and layered structure. The surface of chitosan was improved after the grafting of acrylic acid and acrylonitrile with chitosan. The surface of the cross-linked sample [chit-cl-Poly (AA-co-An)] was uneven non pours and uneven morphology.EDX images of pure chitosan and binary grafted chitosan indicate that both are consist of carbon (C), Oxygen (O), nitrogen (N) atoms.

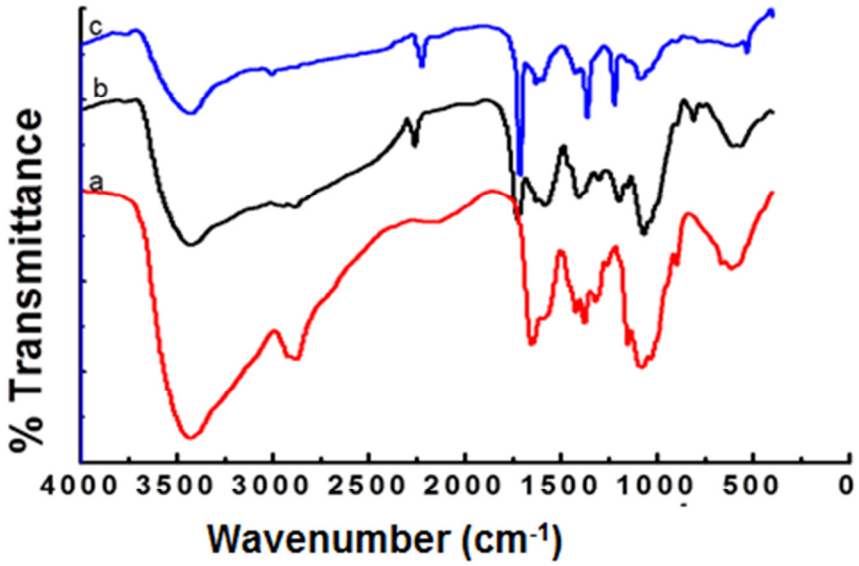


Fig. 27. FT-IR spectra of the (a) chitosan, (b)Chit –cl- Poly (AA-co-An), (c) Chit –g-Poly (AA-co-An).

3.4.4.3.X-Ray analysis

XRD pattern of chitosan, chit-g-Poly (AA-co-An) and chit-cl-Poly (AA-co-An) have been shown in Fig. 29.The pure chitosan showed characteristic peaks at 2θ = 19.64°, which were assigned to be (0 0 1) and (1 0 0) respectively. XRD spectrum of chit-g-Poly (AA-co-A) grafted sample (3b) has a broad characteristic peak at 2θ = 17.92° i.e. the position of the peak at 2θ = 19.64°, in chitosan has been shifted to 17.92°, after the grafting of acrylic acid and acrylamide onto the chitosan. XRD spectrum of chit-cl-Poly (AA-co-A) crosslinked sample is shown in Fig. (29 c) has a broad characteristic peak at 2 θ = 21.07° i.e. the position of the peak at 2θ = 19.64° in chitosan has shifted to 21.07°, after the crosslinking of acrylic acid and acrylamide onto the chitosan. It indicates the crystallinity of chitosan decreases after grafting and cross-

linking. The first stage ranged from 29 to 220 °C (21.8 % weight loss) corresponding to the evaporation of free water.

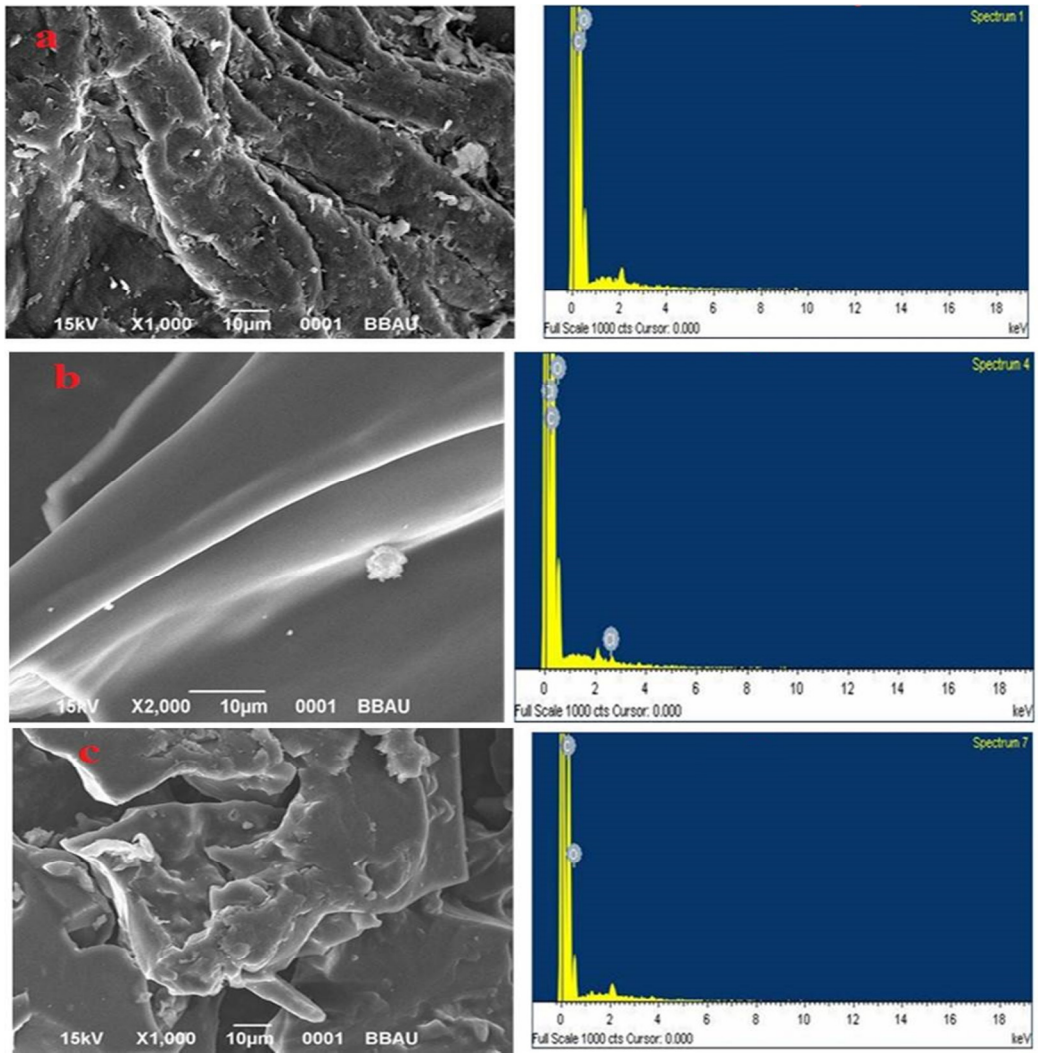


Fig. 28. SEM and EDS images of the (a) chitosan, (b) chit-g-Poly(AA-co-An) (C) chit-cl-Poly(AA-co-An

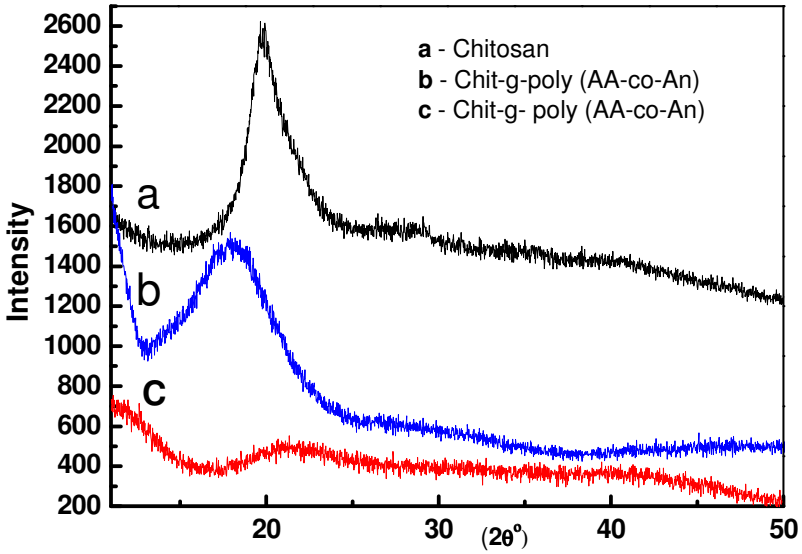


Fig 29. XRD spectra of the (a) chitosan, (b) chit-g-Poly(AA-co-An),(c) chit-cl-Poly(AA-co-An)

3.4.4.4.Thermal study

Thermal behaviour of the chitosan, chit-g-Poly (AA-co-An) and chit-cl-Poly (AA-co-An) are studied by thermal gravimetric analysis and results are displayed in Fig. 30. As shown in Fig.30 a chitosan shows three thermal degradation stages. The initial weight loss of 11.51% (in the first stage) up to temperature 30-200 °C was due to the loss of moisture (water molecule) adsorbed by the polysaccharide [37]. It is significant that the water retains up to temperature of 150 °C is higher than free water (usually 115°C). Strong hydrogen bonding between active groups (amine, hydroxyl) of chitosan and the water molecule is broken at the high temperature. In the second stage, the weight loss is upto 86.79% in the temperature range of 200-528 °C. Decomposition of chitosan started on 230°C and 50% of chitosan loss occurred up to 310 °C. In the third and last stage, the polymer backbone of chitosan chain was completely degraded at 528°C.Chit-g-Poly (AA-co-An) is also showing two thermal degradation stages. In the second stage, the weight loss is 65.49% at the temperature range of 220-524 °C.

Decomposition of chit-g-Poly (AA-co-An) started at 220°C and 50% weight loss of chit-g-Poly (AA-co-An) was observed at the temperature is 320 °C.

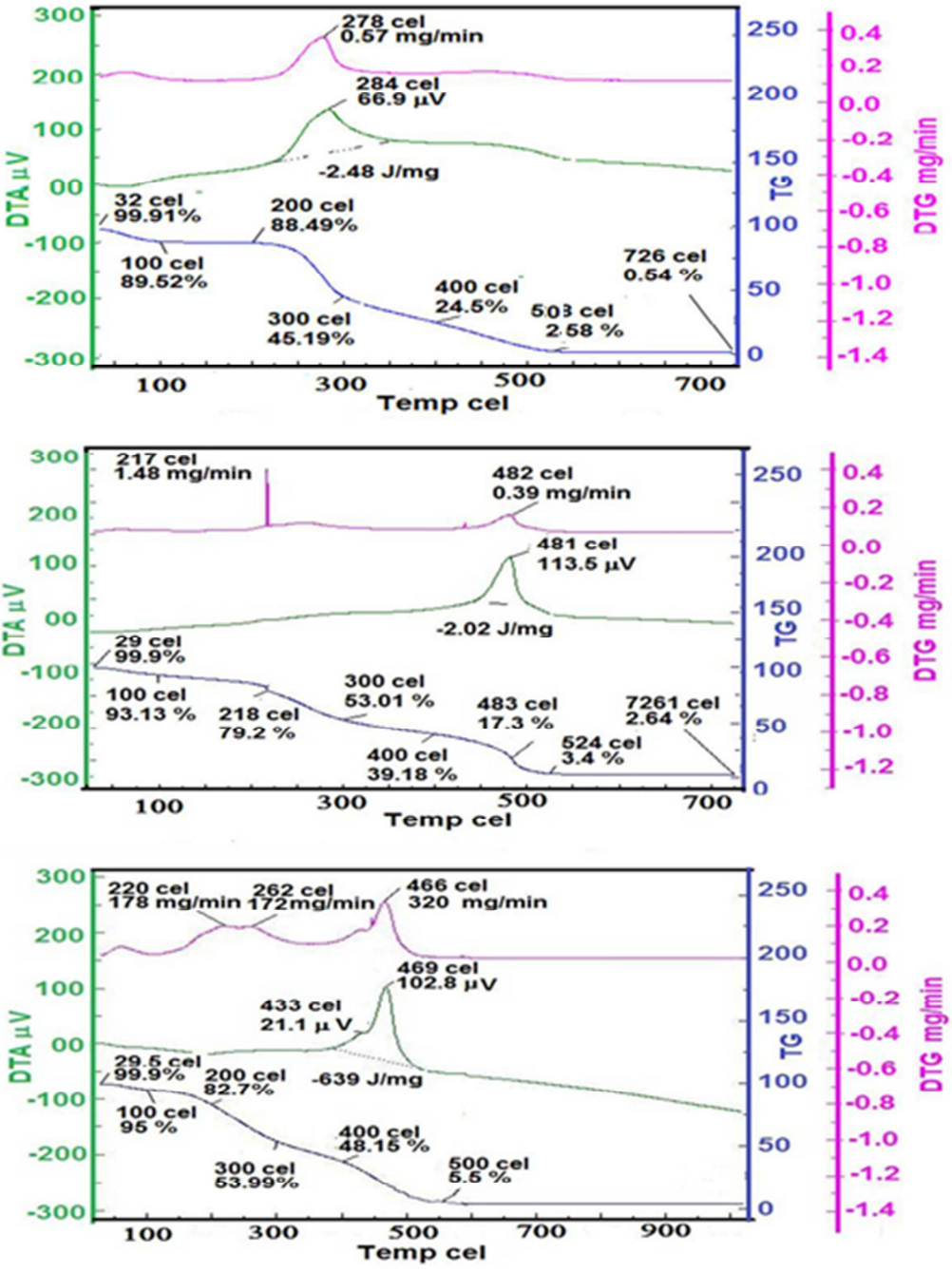


Fig. 30. TGA, DTA and DTG curves of (a) chitosan, (b) Chit-g-Poly(AA-co-An) (c)Chit-cl-Poly(AA-co-An)

Finally, the polymer backbone of chit-g-Poly (AA-co-An) was completely degraded at 550°C which is slightly higher comparison to original chitosan shown in

Fig. 30 b. Chit-cl-Poly (AA-co-An) is also exhibited two thermal degradation stages as shown in Fig 30 c. The first stage ranged from 29 to 220 °C (17.3 %) which corresponds to the evaporation of free water. In the second stage, the weight loss is 77.2% in the temperature range of 220-550 °C. Decomposition of chit-cl-Poly (AA-co-An) started at 200 °C and 50% weight loss of chit-cl-Poly (AA-co-An) occurred at the temperature of 420 °C. Finally, the polymer backbone of chit-cl-Poly (AA-co-An) was completely degraded at 600 °C which is slightly higher as compared to original chitosan and chit-cl-Poly (AA-co-An). It indicates that the thermal stability of chitosan is increased after the grafting/cross-linking of acrylic acid and acrylamide on to the chitosan. It is also evident from DTA and DTG curve where after grafting and crosslinking the product become comparatively thermally more stable in comparison to chitosan, as one major peak in both the case appears at 481 (113.5 uV) in Fig. 30 b and at 469 (102.8 uV) in Fig. 30 c respectively i.e. the grafted material has enough stability up to 525°C while the chitosan itself almost decomposes upto 500°C.

3.4.5. Antibacterial activity of chit-g-Poly (AA-co-An)

Purified grafted and crosslinked copolymers were used for *in-vitro* antibacterial activity. Agar well diffusion method was used for the antimicrobial screening of the plant extracts against the test pathogens. Autoclaved (sterile) nutrient agar media were prepared and poured into sterile petri plates and cultured media was then allowed to solidify. 50 µl of pathogen culture was then spread on to the plates labelled as *Staphylococcus aureus (S. aureus)* MTCC 902, *Escherichia coli (E.coli)* MTCC 1687, *Pseudomonas aeroginosa (P. aeroginosa)* 741. After 3-4 minutes of spreading 4-5 wells of 8 mm diameter were bored using a sterile borer. In each well, 50µl of samples were loaded. Bacterial plates were incubated at 37 °C overnight. After 24hrs checked the zone of inhibition and measured their diameter that shows how much it inhibited the growth of pathogens. Tetracycline was used as a reference drug to estimate the potency of the tested compound under the similar conditions of experiments.

The *in-vitro* antibacterial activity of the compounds [chit-g-Poly (AA-co-An)] and [chit-cl-Poly (AA-co-An)] was studied through the agar culture, including *Escherichia coli Staphylococcus aureus and Pseudomonas aeroginosa*. The results of the antibacterial activities of synthesized compounds [(chit-g-Poly (AA-co-An)] and

[(chit-cl-Poly (AA-co-An)] grafted and cross-linked copolymer exhibited promising effects compared the control marketed antibacterial drugs as shown in Fig. 31. On the other hand, graft copolymer [chit-g-Poly (AA-co-An)] exhibited the highest antibacterial activity against the *Pseudomonas aeroginosa* (31 mm zone inhibition). Furthermore, chit-g-Poly (AA-co-An) graft copolymer also exhibited the better activity against *Escherichia coli* (31 mm zone inhibition) and *Staphylococcus aureus* (26 mm zone inhibition) while crosslinked copolymerchit-cl-Poly (AA-co-An) has shown the highest antibacterial activity against the *Pseudomonas aeroginosa* (36 mm zone inhibition). Furthermore, chit-cl-Poly (AA-co-An) graft copolymer also exhibited the better activity against *Escherichia coli* (27 mm zone inhibition) and *Staphylococcus aureus* (21 mm zone inhibition) as compared to the standard drug tetracycline (24.6 mm zone inhibition). The previously reported results due to the presence of long alkyl moiety in the monomeric unit of the compound are the strong parameter affecting bactericidal performance. However, this observation of similar trend has proved that chit-g-Poly (AA-co-An) and chit-cl-Poly (AA-co-An) copolymers also contained long alkyl moiety in the molecular structure of newly synthesized grafted and cross-linked copolymer. The investigation of high zone inhibition of chit-g-Poly (AA-co-An) and chit-cl-Poly (AA-co-An) [38].

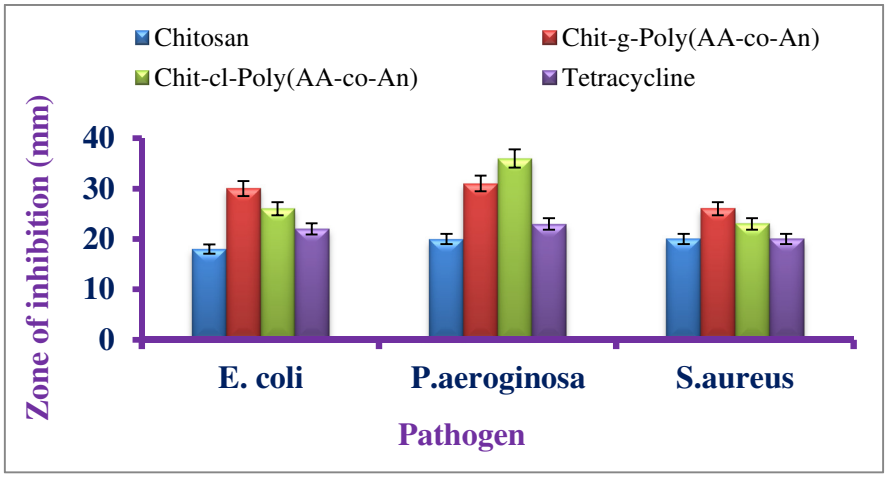


Fig. 31. Antibacterial activity of chitosan, chit-*g*-Poly (AA-co-A) and chit-cl-Poly (AA-co-A) against *E. coli, P. aeroginosaand S. aureus.*

3.5. Conclusions

The synthesis, characterization and antibacterial activities of acrylic acids and acrylonitrile grafted and cross-linked onto chitosan using the microwave irradiation were investigated in the present study. FT-IR confirmed the successful grafting and cross-linking of acrylic acid and acrylonitrile onto chitosan. SEM investigated that the surface morphology of chitosan was improved after grafting and cross-linking. XRD demonstrated that the crystallinity of both grafted as well as crosslinked the products are having amorphous character. Thermal Studies show enhanced thermal stability after grafting and crosslinking, meanwhile cross-linked/grafted chitosan is more stable than pure chitosan. Grafted and crosslinked samples exhibited the excellent antibacterial activities against gram-positive and gram-negative bacteria. Grafted copolymer [chit-g-Poly (AA-co-An)] showed the highest antibacterial activity against the *Pseudomonas aeroginosa* (31 mm zone inhibition). Furthermore, chit-g-Poly (AA-co-An) graft copolymer also exhibited the better activity against *Escherichia coli* (31 mm zone inhibition) and *Staphylococcus aureus* (26 mm zone inhibition) while cross-linked copolymer chit-cl-Poly (AA-co-An) showed the highest antibacterial activity against the *Pseudomonas aeroginosa* (36 mm zone inhibition). Furthermore, chit-cl-Poly (AA-co-An) graft copolymer also exhibited the better activity *against Escherichia coli* (27 mm zone inhibition) and *Staphylococcus aureus* (21 mm zone inhibition) as compared standard drug tetracycline (24.6 mm zone inhibition). *In vitro* antimicrobial activity of the title compound chit-*g*-Poly (AA-co-An) might emerge as lead in the development of potent antimicrobial drugs as well as would be a better polymer for the development of the pharmaceutical doses in order to nanocarrier doses to the bacterial target. However, the polymeric properties of the compound chit-*g*-Poly (AA-co-An) would be a milestone in the path of pharmaceutical to the development of pharmaceutical dosage have great potential as antibacterial food packaging materials.

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Chapter -4

Binary grafted chitosan film: Synthesis, characterization, antibacterial activity and prospects for food packaging

The antimicrobial binary grafted chitosan film [chit-g-Poly (An-co-Am)] was prepared by grafting of acrylonitrile and acrylamide on to chitosan via microwave initiated graft copolymerization. The grafting of acrylonitrile and acrylamide onto chitosan backbone was confirmed by FTIR, XRD, SEM and TGA/DTA/DTG analytical techniques. The binary grafted chitosan film possessed efficient antimicrobial activity against three tested strains, i.e. *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The prepared binary grafted chitosan film was tested for packaging apple and guava to prevent microbial infection and extend their shelf life. The biodegradability study of binary grafted chitosan film was also done and all the results were positive.

Chapter-4 *Binary grafted chitosan film: Synthesis, characterization, antibacterial activity and prospects for food packaging*

4.1. Introduction

Biopolymers and their derivatives are considered as probable eco-friendly substitutes for non-renewable and non-biodegradable plastic wrapping materials. Food packaging protects food from various environmental factors, such as temperature, light, humidity, odours, microorganisms, shocks, dust, vibrations and compressive forces that can lead to degradation [1] and deterioration [2, 3]. New and safe food packaging technologies emerged as a great demand of the civilized society, due to continuous preferences of consumer towards mildly processed, fresh food products with prolonged shelf life and convenience [4]. Furthermore, changing scenario of retail practices (such as global distribution of food) and lifestyle of consumers (resulting in increasing demand of convenient foods such as "ready to cook", "ready to eat" and "ready to use" food) present major challenges to the food packaging industry for the development of new and improved packaging concepts that address such issues in an attractive, safe, durable (extended shelf life) and healthy way; this leads to the emergence of active food packaging [5, 6]. Therefore, the aim of active packaging is to satisfy the consumer demand for natural, recyclable, and biodegradable packaging materials [7].

In the recent years, antibacterial and antifungal packaging emerged as a typical form of active packaging. It has attracted increasing attention in protecting food from foodborne microbial eruptions and in extending the shelf-life with retention of food quality [8, 9]. Applications of biopolymers with antimicrobial activities such as polysaccharides, proteins, and lipids, have been proven to be a favourable approach for food packaging applications because of their environmentally friendly and biodegradable properties [10-13]. Chitosan is a high molecular weight linear cationic hetero polysaccharide composed mainly of β-(1,4)-2-deoxy- 2-amino-D-glucopyranose units and partially of β-(1,4)-2-deoxy-2- acetamido-D-glucopyranose derived from chitin [14] by partly de-acetylation. It shows superior antibacterial and antifungal properties [15-17] and one of the promising polymer for active packaging materials [18], for reasons of its biocompatibility, biodegradability, antioxidants [19], having desirable film forming properties [16], high permeability to gases, and non-toxicity [20]. Grafted chitosan films are an interesting agent in food conservation applications and as antimicrobial candidate in the various industries. In food preservation field, chitosan has various applications such as preservation of fish during storage, to improve the quality of

fresh broccoli, to prevent spoilage of cold pork products and to control bacterial contamination during brewing [21-24]. Many examples are known in which edible chitosan coating increase shelf life and maintains the nutritional quality of vegetables and fresh fruits [25, 26]. Chitosan is also classified as a safe food preservative in the US, EU, and China [27]. However, pure chitosan films were not satisfactory for food packaging applications due to the mechanical properties [28, 29]. Developing active packages based on synthetic polymers and chitosan is not simple because of poor compatibility between the hydrophobic behaviour of synthetic polymer and hydrophilic behaviour of chitosan that might damage the film forming properties of the resultant material.

Further, Foster et al., reported that chitosan films possess almost no antibacterial activity against *E. coli* while chitosan solutions had a remarkable microbial inhibition [30]. Leceta et al. were also found the same results [31]. Conversely, Kim et al. showed inhibition of *S. typhimurium* and *E. coli* [32]. The authors mention that the molecular weight of chitosan, which determines the viscosity, is an important factor for the quality and antibacterial efficiency of the chitosan films. They illustrate this by using chitosan films of different molecular weights. Only chitosan films of lower molecular weights (30 and 90 kDa) seem to be active while higher molecular weight film (100 and 300 kDa) showed no antimicrobial effect [32]. Unfortunately, the molecular weight was not mentioned by Foster et al. [30] whereas Leceta et al. [31] solely refers to low and high molecular weight chitosan without mentioning an actual value. The modification of chitosan films results in an enhanced activity. For example, galic acid-chitosan films showed antibacterial activity towards *E. coli* and *S. typhimurium* [33], and quaternary chitosan films were more efficient towards *E. coli* compared to chitosan films [34]. The applicability of chitosan films in the food and textile sector are comparable for gram (+) and gram (−) bacteria and is already reported by Verlee et al. [26].

Chitosan, as a biodegradable polymer, is the best option suitable for grafting reactions with synthetic monomers. In general, graft copolymerization involves the perturbation of side chains of the polymer without affecting the backbone of the polymer, and therefore, the molecular properties of the backbone are least affected [35]. Furthermore, such copolymers are susceptible to biodegradation too.

The synthesis of graft copolymers can be achieved by following methods:

1. Chemical free radical initiators (conventional method), 2. High energy radiation (gamma rays or electron beam), 3. UV-radiation based methods and 4. Microwave-based methods. High energy radiation method due to their high penetrating power is not suitable for grafting of polysaccharide backbone and UV-radiation based method is restricted only for surfacial grafting [36].

4.2.Earlier method of synthesis

In microwave initiated graft copolymerization, the inert atmospheric condition is not necessary unlike the case of the conventional method of synthesis of the graft copolymer (CAN initiated method). Microwave initiated methods are fast, clean, eco-friendly, easy to operate, highly reproducible and therefore, the most suitable method of synthesis [37].

A large number of synthetic methods have been developed to prepare the chitosan-based film. Solution casting method is the significant method to prepare the polymeric film from natural polysaccharides.

Zhai et al. have prepared starch/chitosan blend films by irradiation methods. They found that the flexibility and tensile strength of the starch film were enhanced after incorporation of 20% chitosan into the starch film. The antibacterial activities of starch/chitosan blend film was measured by optical density method, against the *Escherichia coli* (E. coli) [38].

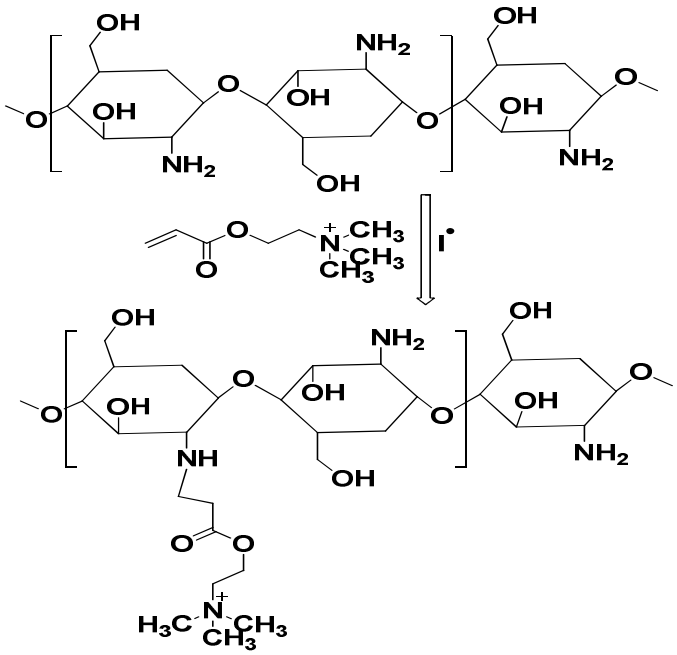
Khan et al. have prepared chitosan/starch blend film by mixing an aqueous solution of starch (1%) with chitosan (chitosan: starch=1:2, w/w). They achieved the elongation at break (%Eb) and tensile strength (TS) of chitosan/starch blend 3.6% and 9.33 MPa, respectively [39]. Similarly Xu et al. have prepared chitosan/starch composite films by combining chitosan (deacetylated degree, 90%) solution and two thermally gelatinized corn-starch (waxy starch and regular starch with 25% amylose). They have studied the tensile strength (TS), water vapor transmission rate (WVTR) and elongationat break (%E) of the film [40].

Shankar et al. have synthesized the chitosan-based film by using a solution casting method for food packaging, they found that the addition of sulfur nanoparticle improved the mechanical strength, hydrophobicity, and antimicrobial activity of the chitosan film. This film has the strongest antimicrobial activity against food-borne pathogenic Gram-negative (*Escherichia coli*) and Gram-positive (*Listeria monocytogenes*) bacteria [41].

Sokolova et al. synthesized the chitosan-based film via solution casting method by using the deep eutectic solvent based on malonic acid and used choline chloride as a plasticizing agent [42].

Riaz, et al. synthesized the chitosan-based antimicrobial active food packaging film incorporated with apple peel polyphenols (APP). They have used glycerol as a plasticizer. They have studied the physical properties of this film such as moisture content, opacity, color, density, swelling ration water vapor permeability and water solubility and observed that the addition of APP into chitosan significantly improved the physical properties of the film. It was found that the tensile strength and elongation at break of the film was 16.48 MPa and 13.33%, respectively [43].

Hassan et al, have prepared the chitosan films graft copolymerized with poly (acryloyloxy) ethyltrimethylammonium chloride. Firstly they synthesized the graft copolymer of chitosan with poly (acryloyloxy) ethyl trimethylammonium chloride via the redox polymerization (scheme 1) after that they prepared the film through a solution casting method. Antimicrobial activity of this film was investigated against two fungi (*Aspergillus brasiliensis* and *Aspergillus fumigatus*) and three bacteria (S*taphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*). They found that the antibacterial activities of grafted chitosan film were higher than pure chitosan [44].



Scheme 1. Schematic representation of grafted chitosan.

Santonicola et al. synthesized the chitosan-based film with methylcellulose containing natamycin (antimicrobial) by casting process for food packaging application. They investigated the antimicrobial's release of the films in 95% ethanol (*v*/*v*) at different temperatures and found that the release of natamycin from methylcellulose film ($D_P = 3.20 \times 10^{-8}$ cm^2/s was more as compared to pure chitosan-based film ($D_P = 3.61 \times 10^{-13}$ cm^2/s) at the same temperature [45]. Siripatrawan et al. prepared the chitosan and glycerol-based film by casting method which was used as an active packaging [19, 46]. Ashrafi et al. have prepared the biocomposite film based on chitosan and kombucha tea for food packaging by a solvent casting process. The antimicrobial activity of this film was evaluated against *Escherichia coli* and *Staphylococcus aureus* via agar diffusion method, and its antioxidant activity was also determined through DPPA assay method. It was observed that grafted chitosan film has the high antimicrobial and antioxidant activity as compared to pure chitosan [47].

4.3.Basis of work

Chitosan and its derivatives are considered as renewable, degradable and environmentally friendly materials. Chitosan-based films have an interesting application in food preservation and used as antimicrobial candidate in the several industries. The chitosan-based food packaging materials maintain the quality and safety of food products during storage/transportation and expand their shelf-life by preventing unfavourable factors such as spoilage microorganisms, chemical contaminants, oxygen, moisture, temperature and external force. Keeping the above fact in mind, we hereby proposed the synthesis of the binary grafted chitosan-based film for food packaging.

4.4. Present work

The current research aimed to develop, synthesize and characterize a new antibacterial and biodegradable binary grafted chitosan film by microwave irradiation without using any initiator. The influence of monomers concentration, microwave power and reaction time, in the reaction mixture on the percentage of grafting is investigated. Our hypothesis behind this work was that the binary grafted chitosan film will show greater moisture holding capacity and mechanical strength due to the uniform dispersion of acrylamide and acrylonitrile on chitosan. We report the synthesis, thermal behavior, and antibacterial and biodegradable properties of binary grafted chitosan film and verify that the antibacterial properties of this new graft copolymer are not hampered, compared to inherent chitosan.

4.5.Experiment

4.4.1Material

Chitosan (viscosity < 200 cps, molecular weight~40 kDa, deacetylation 80%) was purchased from Jaipur Scientifics and Chemicals Bareilly, India (Otto Chemie Pvt Ltd, Mumbai, India). Acrylamide (Am), acrylonitrile (An), methyl alcohol (MeOH), acetone (MeCOMe) and hydroquinone were all supplied by E. Merck Ltd. Mumbai, India. All experiments were carried out on a domestic microwave (LG. MH 2548QPS).

4.4.2. Synthesis of binary grafted chitosan films

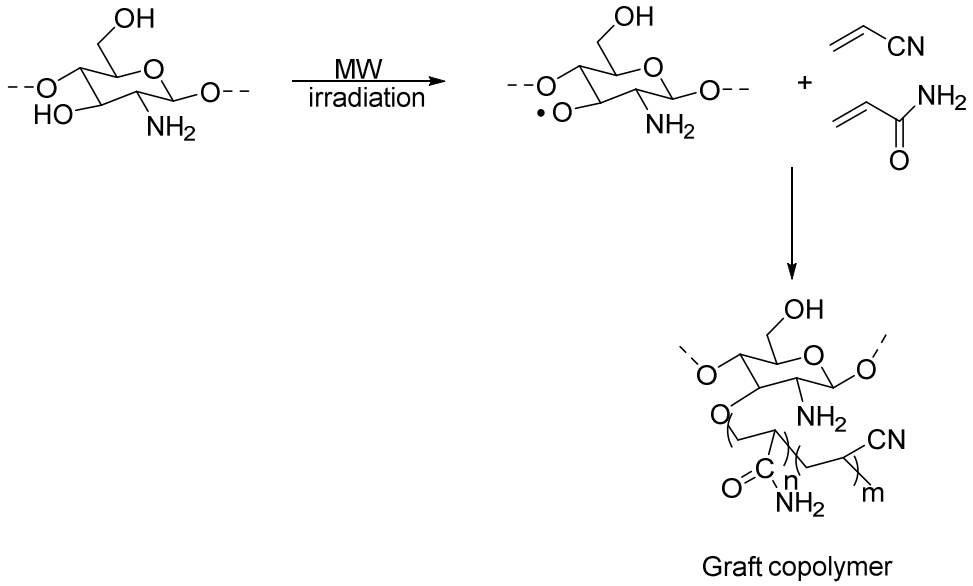
1g of chitosan was dissolved in 100 mL double distilled water (1% acetic acid) with constant stirring. Desired amount of acrylonitrile and acrylamide were added to chitosan solution and stirring was continued for further 30 minutes to get homogeneous mixture [48]. The homogeneous mixture containing chitosan, acrylamide and acrylonitrile was irradiated with microwave radiation (80% power) for 90 seconds in domestic microwave oven, after which it was terminated by adding a saturated solution of hydroquinone. At the end of the reaction, two layers were formed (one layer contain homopolymer and other layer contain desired graft copolymer) which were separated with the help of separating funnel. The layer containing graft copolymer was spread on glass plate and dried in oven at 40 °C to obtain the film. The reaction was studied for optimal grafting at different, monomer concentration at microwave power 80 % (1635 W) and exposure time 90 seconds [49, 50].

The % grafting of this microwave synthesized binary grafted chitosan film was calculated by the Eq. (1)

$$\%\ \text{Grafting} = \frac{\text{wt. of grafted film} - \text{wt. of polysaccharide}}{\text{wt. of polysaccharide}} \text{x}100 \ldots\ldots\ldots \text{Eq. (1)}$$

PAN and PAAm were simultaneously grafted onto chitosan in a homogeneous medium using microwave (MW) irradiation as a radical initiator. The proposed mechanism of copolymerization of An and AAm onto chitosan in the presence of MW irradiation is shown in Scheme 1. The MW irradiation generates alkoxy radical from the polysaccharide substrate and resulted in active centers on the substrate to radically initiate polymerization of AN and AAm which led to graft copolymer [51, 52].

The optimum reaction conditions were determined by the variation of one of the reaction parameters (monomer concentration, chitosan concentration, microwave power reaction temperature and reaction time), while other parameters were kept constant.



Scheme 1 Proposed mechanism for radical graft polymerization of acrylamide and acrylic acid onto chitosan in the presence of microwave irradiation.

4.4.3. Influence of reaction parameters on grafting

4.4.31. Effect of acrylamide concentration

The effect of acrylamide monomer concentration [M1] on grafting was studied by changing it from 42.0 x 10^{-3} to 168.0 x 10^{-3} mol L^{-1} while keeping the other reaction parameters constant such as acrylonitrile concentration 7.0 x 10^{-3} mol L^{-1}, microwave power 1635W and time 90 sec. The results are illustrated in fig.32a, and it showed that % grafting was found to increase on increasing monomer concentration from 42.0 x 10^{-3} to 126.0 x 10^{-3} mol L^{-1} to a maximum value of 140%. Availability of a large amount of monomer during chain propagation step in grafting copolymerization led to increase in %G. However, on further increase of monomer concentration (168.0 x 10^{-3} mol L^{-1}) leads to decrease in percentage grafting (135%). This is probably because of increase in the viscosity of the medium, which opposes the diffusion of the monomer towards the chitosan, and consequently the residual monomers are consumed with the formation of homopolymer.

4.4.3.2. Effect of acrylonitrile concentration

The effect of acrylonitrile monomer concentration [M2] on grafting was studied by changing it from 7.0 x 10^{-3} to 17.5 x 10^{-3} mol L^{-1} while keeping the other reaction parameters constant such as acrylamide concentration 126.0 x 10^{-3} mol L^{-1}, microwave power 1080W and time (90 sec). The results are illustrated in fig. 32b and they showed that % grafting was found to increase on increasing monomer concentration from 7.0 x 10^{-3} to 14.0 x 10^{-3} mol L^{-1} to a maximum value of 156%. However, on further increase of monomer concentration (17.5 x 10^{-3} mol L^{-1}) results in decrease in % grafting (147%) with increase in formation of homopolymer.

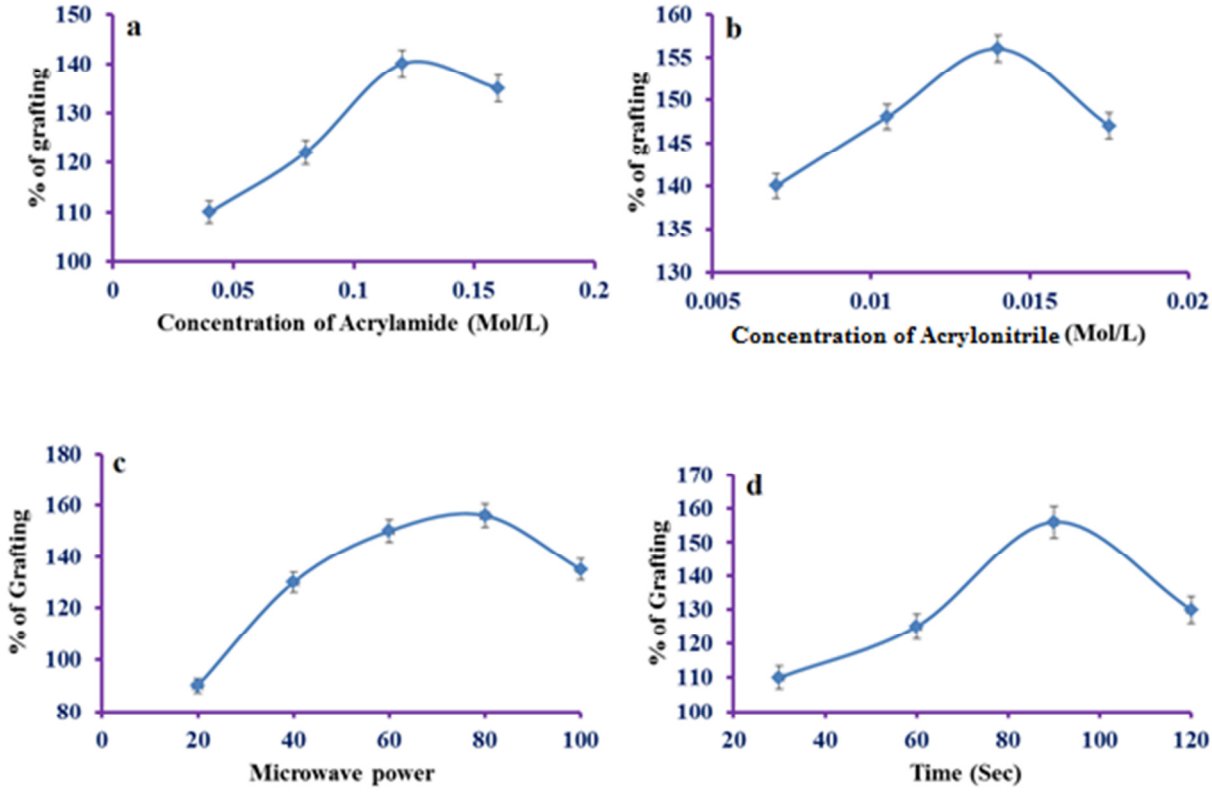


Fig. 32. Effect of various parameters on grafting (a) concentration of acrylamide (b) concentration of acrylonitrile (c) microwave power (d) time.

4.4.3.3.Effect of microwave power

The microwave power affected %G was studied, keeping all the other reaction parameters constant such reaction time was 90 s, [Chitosan] was 1g, [M1] was 126.0 x 10^{-3}

mol L^{-1} and [M2] was 14.0 x 10^{-3} mol L^{-1}). Results are shown in fig 32c, which indicates that % grafting was increased on increasing microwave power from 270 W to 1080W, however, further increase in microwave power results in decrease in % grafting.

4.4.3.4.Effect of reaction time

The effect of reaction time was studied, keeping all the other reaction parameters constant such as microwave power was 1080W, [Chitosan] was 1g, [M1] was 126.0 x 10^{-3} mol L^{-1} and [M2] was 14.0 x 10^{-3} mol L^{-1} and results are shown in Fig 32 c, which clearly revealed that % grafting was found to increase on increasing reaction time from 30 s to 90 s to a maximum value of 156%, but on further increase in reaction time %G starts to decrease.

4.4.4. Characterization of the binary grafted chitosan film

4.4.4.1.FTIR

FTIR spectra of chitosan and binary grafted chitosan film are shown in Fig 33. Chitosan showed a broad absorption band in the range of 3500-3000 cm^{-1} (O-H and N-H stretching), 2879 cm^{1} (C-H stretching), 1652 cm^{-1} (Amide I), 1420 cm^{-1} (–NH_2 bending, Amide II) and 1377 cm^{-1} (–CH_2– bending). The absorption bands at 1151 cm^{-1} (anti-symmetric stretching of the C-O-C group), 1060-1020 cm^{-1} (skeletal vibrations involving the C-O stretching) are common in both spectra because of the chitosan backbone [52]. On the other hand, FTIR spectra of the binary grafted chitosan film show a characteristic band at 2240.78 cm^{-1} (C≡N stretching of nitrile), 1595.2 cm^{-1} (C=O stretching of amide-I) 1385.56 cm^{-1} (N-H in plane bending of amide-II) and 1343.48 cm^{-1} (C-N stretching of amide-III) (Fig 33b) which shows quite resemblance with earlier reported IR data of graft copolymer of chitosan [53] and hence clearly indicates the grafting of acrylonitrile and acrylamide on chitosan under microwave irradiation.

4.4.4.2.SEM and EDX

The surface morphology (SEM) and EDX images of chitosan and binary grafted chitosan film [chit-*g*-Poly (An-co-Am)] are shown in Fig 34. The images revealed changes in surface morphology of chitosan and binary grafted chitosan film. The surface of pure chitosan showed irregular morphology as porous heterogeneous rough and layered surface with various types of flakes as shown in Fig 34a, that was changed to a smooth surface in grafted copolymer sample (Fig 34b). Therefore, the surface morphology supported the grafting of acrylonitrile and acrylamide on to the chitosan side chains. EDX images of pure

chitosan and binary grafted chitosan film indicate that both are consist of carbon (C), Oxygen (O), nitrogen (N) atoms.

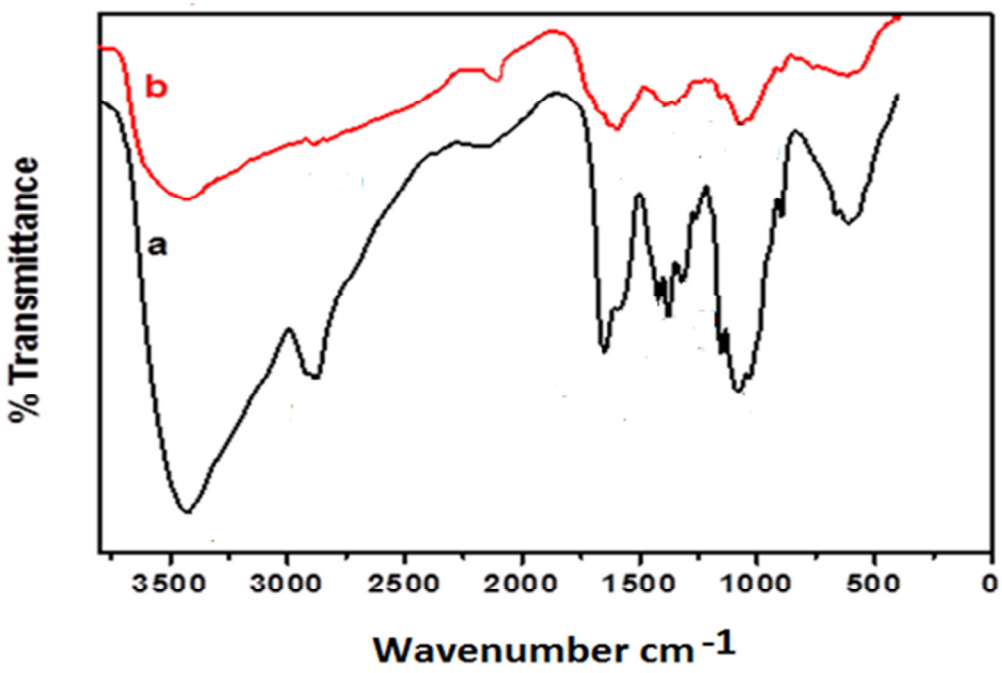


Fig. 33. FTIR spectra (a) pure chitosan (b) binary grafted chitosan film.

4.4.4.3.XRD

After grafting, changes in the chitosan structure were further investigated by means of X-ray diffraction. The XRD spectra obtained for a pure chitosan and binary grafted chitosan film [chit-*g*-Poly (An-co-Am)] are shown in Fig 35. Pure chitosan (Fig 35a) is characterized by one scattering angle at 2θ = 20° which indicates chitosan is an anhydrous crystal. On the other hand, for the XRD spectra of binary grafted chitosan film (Fig 35b) it can be observed a decrease in the intensity of the peak at 2θ = 22° and a shift of the maximum of this peak to 2θ = 22°. The lower intensity indicates the decrease in crystallinity due to incorporation of acrylamide and acrylonitrile along the biopolymer backbone which is attributed to the grafting process.

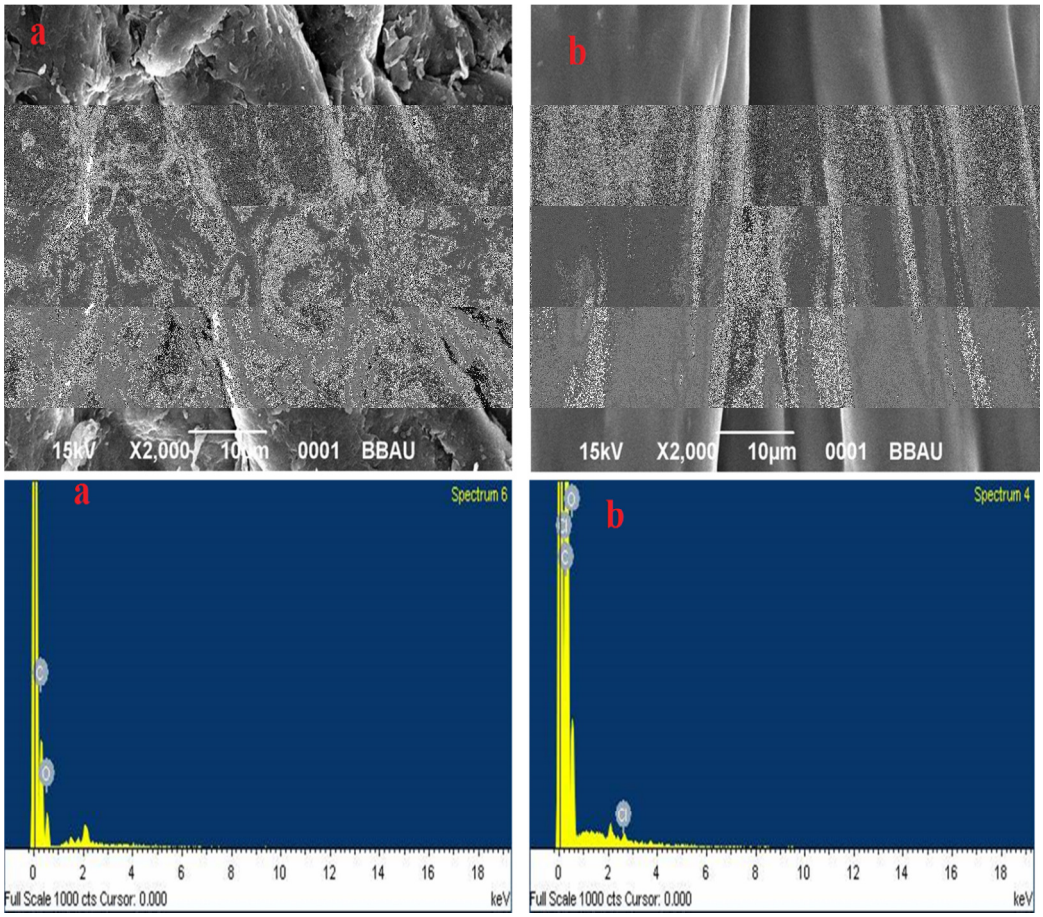


Fig 34. Scanning electron micrograph and EDX of (a) pure chitosan (b) binary grafted chitosan film.

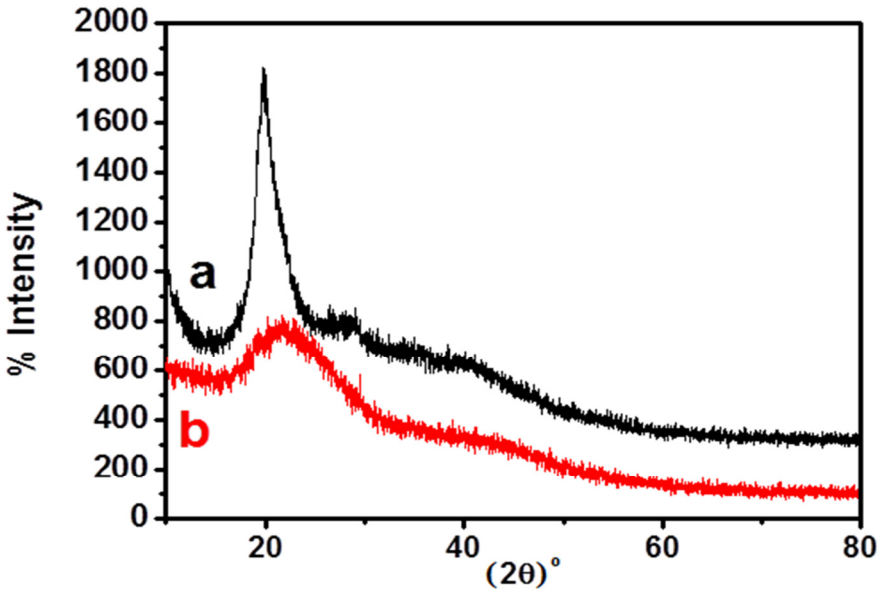


Fig. 35. XRD spectra (a) pure chitosan (b) chit-g-Poly (An-c-Am).

4.4.4.4.Thermal behaviour of chitosan and binary grafted chitosan film [chit-g-Poly (An-co-Am)]

Thermal degradation of pure chitosan and binary grafted chitosan film was studied by means of TGA, DTA and DTG curves. Fig 36 displays curves in the form of weight loss as a result of controlled heating (versus temperature) for pure chitosan and binary grafted chitosan film. TGA characteristics parameters are:

1. T_{onset}, temperature at which thermal degradation starts
2. T_{peak}, temperature at which thermal degradation is maximum
3. T_{endset}, temperature at which thermal degradation is complete and
4. W%, is the percentage of weight loss during each stage

The comparative TGA data of pure chitosan and binary grafted chitosan film are shown in Table-6.

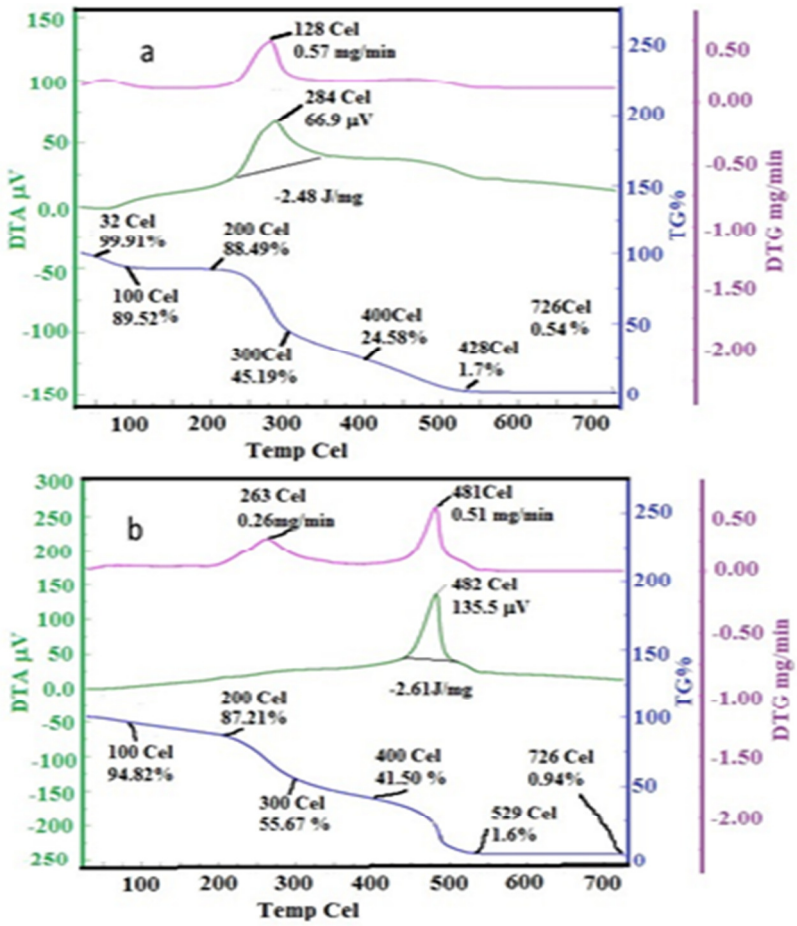


Fig. 36 TGA, DTA and DTG curves of (a) pure chitosan (b) binary grafted chitosan film

The decomposition curves of pure chitosan were divided into 3 stages. The first stage ranging between 32 -200 ºC is attributed to the loss of crystal water and melting of amorphous structure of chitosan. The second stage between 225 -300 ºC with maximum decomposition temperature at 284 ºC referred to dehydration of the saccharide rings and depolymerization of pure chitosan whereas the third stage between 300-428 ºC clearly indicates the complete decomposition of pure chitosan.

The binary grafted chitosan film was also degraded in three distinguished steps. The first step lied between 29-200 ºC with a small weight loss of 12.78% referred to the loss of water. The second step involves the weight loss of 45.71% in temperature range of 200-400 ºC which showed a maximum weight loss occurred at about 300 ºC, indicating a better thermal stability of binary grafted chitosan film in comparison to pure chitosan. The increase in thermal stability was due to acrylamide and acrylonitrile which was successfully grafted on chitosan and changed its molecular structure. The third stage between 400-529 ºC corresponds to a weight loss of about 39.90% which involves the complete decomposition of binary grafted chitosan film.

Table 6:- Thermal parameters obtained from thermogravimetric analysis of pure chitosan and binary grafted chitosan film [chit-g-poly (An-co-Am)].

Material	Stage	T_{onset} (°C)	T_{peak} (°C)	T_{endset} (°C)	W%
Pure Chitosan	I	32	200	100	11.42
	II	225	300	284	39.30
	III	300	428	400	47.49
Binary Grafted Chitosan film	I	29	200	100	12.78
	II	200	400	300	45.71
	III	400	529	481	39.90

4.4.5. Antibacterial activities

The antimicrobial activity of binary grafted chitosan film was evaluated against three different pathogenic bacteria, Gram-positive bacteria as *Staphylococcus aureus (S. aureus)* MTCC 902 and Gram-negative bacteria as *Escherichia coli (E. coli)* MTCC 1687,

Pseudomonas aeroginosa (P. aeroginosa) 741. Antibacterial test was carried out using the agar well diffusion method [30]. Diameter of the well was 8 mm and the concentration of the investigated sample was 50 µL. The plates were incubated at 37 °C for 24 h. After incubation, antibacterial activity was estimated by measuring the inhibition zones diameter against the tested organisms. Inhibition diameter zones of antibacterial activity were expressed in millimeters (mm). Tetracycline was used as a reference drug to estimate the potency of the tested compound under the similar conditions of experiments [54].

Antibacterial activity of chitosan and best grafted binary chitosan film (%G 156) were investigated against three pathogenic bacteria that cause foodborne diseases namely *S. aureus* (as gram-positive bacterium) and *E. coli* and *P. aeruginosa* (as gram-negative bacterium) using the agar well diffusion method. The results of antibacterial activity for pure chitosan and binary grafted chitosan film [chit-g-poly (An-co-Am)] are tabulated in Table-7. It is evident from table-2 that pure chitosan exhibited less antibacterial activity than tetracycline (reference drug) and binary grafted chitosan film against all the three strains of pathogenic bacteria whereas binary grafted chitosan film exhibited an increase in antibacterial activity against all the three strains of pathogenic bacteria. This clearly indicates that grafting of acrylamide and acrylonitrile on chitosan increase the antibacterial activity of binary grafted chitosan film.

Table 7:- Antibacterial activity of pure chitosan and binary grafted chitosan film [chit-g-poly (An-co-Am)].

S.N.	Pathogen	Zone of inhibition (mm)		Tetracycline (Reference drug)
		Chitosan	chit-g-Poly (An-co-Am)	
1.	*E. coli*	18±0.5	24.5±0.5	24.2
2.	*P. aeruginosa*	16±0.5	26±0.5	24.5
3.	*S. aureus*	18±0.5	26±0.5	24.6

4.4.6. MTT Assay

MTT assay was used to evaluate cytotoxic activity. In this study, absorbance was used to evaluate the cytotoxic effect of binary grafted chitosan film on A 549 lung cancer cells and determines the percentage of cell viability after 65 hrs. In brief, $1x10^6$ cells were seeded into

24 well plates and experiment started after 70-80% confluence of cells. Different wells were treated with media; acetic acid and chitosan film solution (1 ml of 0.5% acetic solution was used to dissolve 1 mg of binary grafted chitosan film, thereafter, 150 µL of chitosan film solution was added into each well). After 65 hours of incubation, cells were tested for viability using MTT assay according to standard protocol. In brief, 30 µL 3- (4, 5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) solution was added into each well and plate was incubated for 3 hours at 37 °C. After incubation insoluble formazan was dissolved by adding 300 µL solubilizing solution and gently mixed by shaking. For the negative control all reagents were taken without cells and absorbance was taken at 570 nm. All the experiments were performed in triplicates.

Fig. 37 presents the results of the tests determining the percentage of surviving cells after 65 hrs. From the results of MTT assay, there is a clear decrease in % of cell viability in a well containing binary grafted chitosan film solution in acetic acid as well as in a well with 0.5% acetic acid alone. As the film was dissolved in acetic acid (0.5%) solution which itself shows the decrease in % of cell viability drastically therefore, it does not provide the clear results and the further studies are underway.

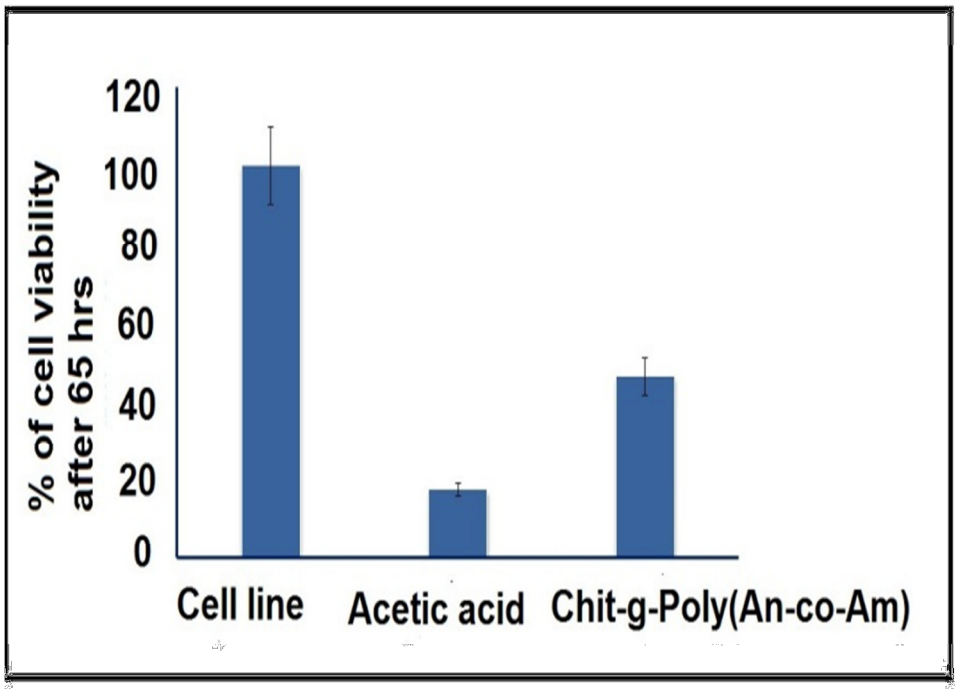


Fig. 37 MTT assay of chit-g-poly (An-co-Am)

4.4.7. Soil burial degradation evaluation

Soil burial degradation test was carried out as reported in the literature [55]. Small pieces of samples (dimensions 7 cm × 7 cm) were buried in natural soil at a depth of 10 cm in Rohilkhand University, Bareilly Campus, India. The average environmental temperature was 40 ± 5 ºC and the relative humidity (RH) was 65–70%. After 15 days and 30 days, the samples were collected, washed with distilled water several times and dried in the oven at 50 °C for 24 h.

The weight loss was calculated using Eq. (2) [56, 57].

$$\text{Weight loss } (\%) = \frac{\text{Initial wt. of sample} - \text{Final wt. of sample}}{\text{Initial wt. of sample}} \text{x}100 \ldots\ldots \text{Eq. (2)}$$

Where, $w_{initial}$ and w_{final} was the weight of the sample before and after soil burial degradation test respectively.

The IR spectra of initial and degraded copolymer films were recorded in a Perkin-Elmer Spectrum 2 FTIR spectrometer under dry air at room temperature (35-40 ºC).

Fig. 38a, presents the weight loss of the best binary grafted chitosan film (%G 156) during 20 days of soil burial test. Furthermore, zero time binary grafted chitosan film was thin and transparent which as time progressed become off-white and opaque. The loss in weight of binary grafted chitosan film is a clear reflection of the biodegradation process performed by the microorganism and moisture present in the soil. The weight loss of binary grafted chitosan was 40% after 12 days of soil burial which was reached up to 80% after 20 days of soil burial (Fig 38b). The weight loss was accompanied with change in molecular structure of the film. As can be observed in Fig 38c, the IR spectra of the binary grafted changed after the soil burial test for 12 and 20 days respectively.

IR spectral data of binary grafted chitosan film showed peak absorption around 3400-3500 cm^{-1} and at 1650 cm^{-1} which undergoes a progressive decrease in intensities as biodegradation takes place. These results clearly indicate that binary grafted chitosan film is biodegradable in nature.

4.4.8. Food preservation evaluation

The application of the prepared binary grafted chitosan film in food preservation as antimicrobial packaging material was studied using apple and guava purchased from a local fruit market. Apple and guava samples were singly packed in binary grafted chitosan film. All the samples were stored at ambient temperature.

In order to evaluate the potential application of the prepared binary grafted chitosan film in food packaging industry, the preservation of apple and guava was studied by wrapping them with binary grafted chitosan film and one pair was left without application of film (as control). It is evident from Fig. 39a-e that apple and guava packed with antimicrobial binary grafted chitosan film were still red and green without decomposition and the surface maintained smooth without any leakage and microbial attack even after 18 days of storage at 40-45 ºC. Furthermore, the pair of apple and guava which was left as control changed to dark reddish-brown in colour containing several moldy spots with a foul smell. This clearly illustrates the satisfactory preservation effect of the binary grafted chitosan film for food packaging application.

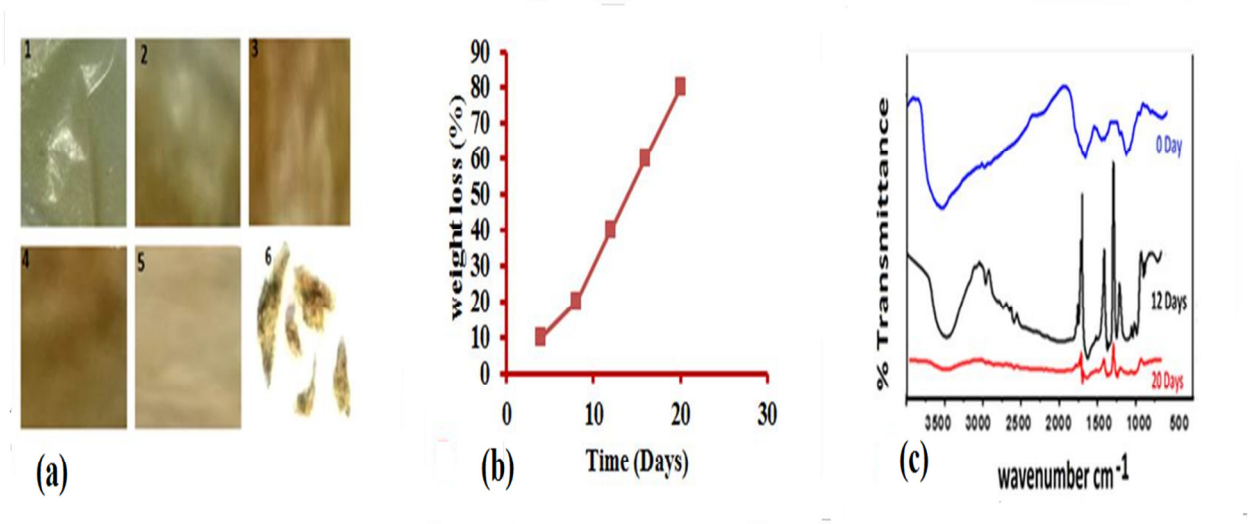


Fig. 38 (a) Soil burial test of binary grafted chitosan film (1) 0 days (2) after 4 days (3) after 8 days (4) after 12 days (5) after 16 days (6) after 20 days. (b) Weight loss of binary grafted chitosan film after the soil burial test for 0, 4, 8, 12, 16 and 20 days. (c) IR spectra of 0 day and biodegraded (day 12 and 20) binary grafted chitosan film.

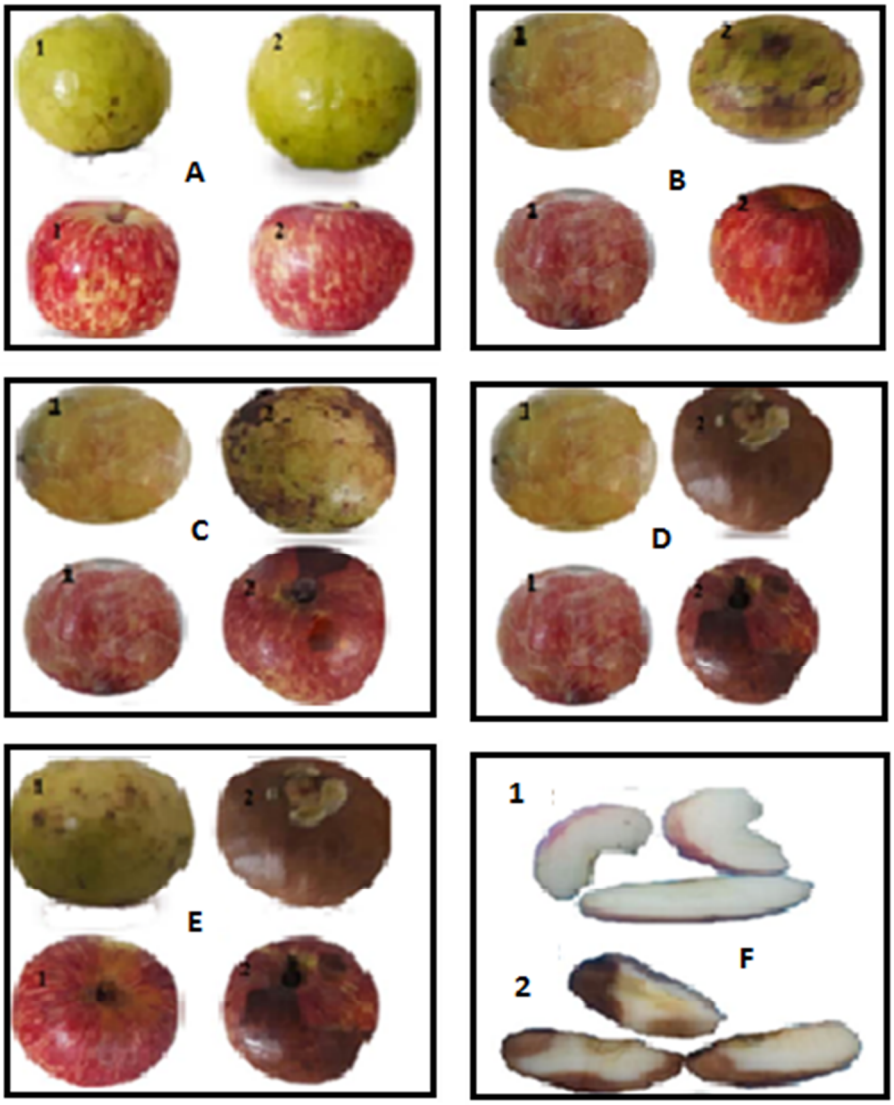


Fig. 39 Food preservation application of antimicrobial binary grafted chitosan film to apple and guava (fruit 1 covered by film and 2 without any film as control) (A) 0 day (B) after 6 days (C) after 12 day (D) after 18 days with film (E) after 18 days without film (F) after 18 days pieces fruit.

4.5. Conclusions

Binary grafted chitosan film [chit-g-Poly (An-co-Am)] with acrylamide and acrylonitrile was synthesized successfully by microwave initiated method and the proposed structure of binary grafted chitosan film was characterized by FTIR, XRD, SEM and TGA/DTA/DTG techniques. The XRD supports the non-crystalline nature of newly synthesized binary grafted chitosan film. TGA analysis illustrated that binary grafted chitosan film was more thermally stable than pure chitosan at elevated temperature. On the other hand,

SEM images referred a change in surface morphology from irregular shape as in pure chitosan to smooth surface in binary grafted chitosan film. The antibacterial activity of pure chitosan and best grafted binary chitosan film was done against three strains of pathogenic bacteria and results showed that binary grafted chitosan film have increased antibacterial activity than pure chitosan and reference drug tetracycline. The soil burial test of binary grafted chitosan film showed that film is biodegradable. Therefore, the prepared binary grafted chitosan film could successfully protect apple and guava from microbial attack and extend their shelf life and is biodegradable in nature. The present paper presented a novel binary grafted chitosan film for potential application in food packaging industry with satisfactory antimicrobial activity and biodegradable nature.

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Chapter -5

Synthesis and characterization of binary grafted material of psyllium for mercury removal

Psyllium-g-Poly-(acrylamide-co-acrylonitrile) has been synthesized from psyllium under N_2 atmosphere, in presence of ceric ammonium nitrate and ascorbic acid couple (CAN/AA) as initiator for adsorption of mercuric ions from synthetic solution of $HgCl_2$. The synthesized samples were optimized by varying synthetic parameters *viz.* monomer concentration, reaction time, temperature, initiator concentration etc. to obtain maximum yield of grafted product as well as maximum adsorption of ionic mercury. The optimized sample has been characterized through FTIR spectroscopy, SEM analysis, X-Ray diffraction and thermal studies (TGA/DTA/DTG). The mercury adsorption was studied onto the optimized sample, and found maximum at temperature (30°C), dose (30 mg), pH (6), time (60 min) and initial concentration of mercury with 100 ppm. Equilibrium isotherm data were analyzed through Langmuir and Freundlich isotherms. Langmuir model was more fitted (R^2=0.9976) which indicated the monolayer sorption. The kinetics of sorption of mercury (II) were also analysed using the first order (R^2= 0.9971), second order (R^2= 0.9887), pseudo-first order (R^2= 0.9971), pseudo-second-order (R^2= 0.9481), intra-particle diffusion (R^2= 0.9958) and Elovich equation (R^2=0.9624). Second order rate kinetics has best linearly fitting, which follows chemisorption mechanism.

Chapter-5 *Synthesis and characterization of binary grafted material of psyllium for mercury removal*

5.1. Introduction

The mercury removal from the water system is a great challenge for the scientific community in past few decades. Mercury is highly concerned in current time because of its toxicity and volatility into the environment [1-4]. Furthermore mercury is declared as one of the significant harmful material by the Agency for Toxic Substances and Disease Registry (ATSDR) because of its toxicity, mobility and long residence time in the atmosphere [5]. The minor concentration of the mercuric ions is toxic to the atmosphere and human health (maximum permissible limit recommended WHO 2.0 μg/L) [6]. The toxicity of mercury mainly depends on its valance state [7].

Mercury in natural water occur in three oxidation states (0, +1 and +2) and may be present in various forms depending on factors such as ionic strength, the concentration of the suspended particulate matter (SPM), temperature, pH, salinity and dissolved organic carbon (DOC). The different forms of mercury include organic mercury (CH_3Hg and C_2H_2Hg), inorganic mercury ($HgCl_2$) and elemental mercury [Hg (0)] [7-9]. Mercury in +2 oxidation state (Hg (II) is most toxic, it has high tendency to bind with protein chains due to the formation of strong Hg–S bond with cysteine residue. It also causes serious damage to the central nervous system, kidney, cardiovascular system, and bones [10, 11]. The main sources of Hg (II) in the environment are various chemical industries mainly chloro alkali, fertilizers, pulp and paper, battery manufacturing, plastic, paint and oil refining etc. [12], mercury reaches in the environment through the waste of these chemical industries [13-15]. However, when vapour of mercury reached into the blood stream via respiratory system, then it is distributed all over the cells in the body and oxidized to more toxic Hg (II) [11, 16].

Infants and children are most affected by mercury toxicity. Methyl mercury affects more than 630,000 new-born each year, by interrupting the development process of their brain and nervous system [17-19]. Deficits in cognitive thinking, memory, language and visual-spatial skills have all been linked to children exposed to methyl mercury in the womb, therefore doctor gives advice to the women do not eat the fish, while they are pregnant because mercury comes from foods, especially fish and seafood [19, 20]. In fishes, elemental mercury is naturally transformed into methyl mercury, a

compound of great toxicity. The vegetables and fruits, especially rice grown in the polluted area is found to store mercury in the form of methyl mercury (34-210 µg/kg), since bacteria convert mercury into methyl mercury by the methylated process [21-23]. Therefore it is necessary to remove the mercury ions from industrial waste and waste water. Many methods have been used for wastewater treatment and heavy metals removal from aqueous solutions such as coagulation, ion exchange, advanced oxidation, reverse osmosis, adsorption, chemical precipitation [16, 24], absorption and biomass adsorption etc. [25, 26]. Among these techniques, the adsorption method is most versatile and widely used for removal of mercury ions from aqueous solutions [27]. Many adsorbents including graft copolymer/modified polymers have been established for heavy metal removal purpose, due to their unique chemical, mechanical, electrical, thermal and rheological characteristics [28].

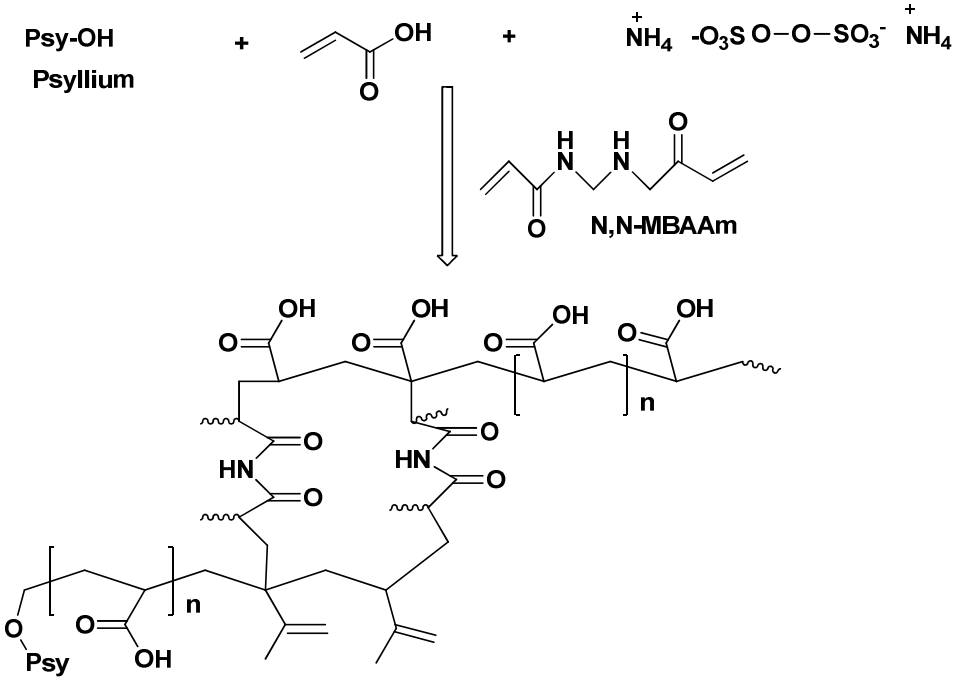
Currently the developments of natural polysaccharide based adsorbent for removal of toxic metal ion from the industrial waste are of great interest [29]. Chitosan and its derivatives act an excellent adsorbent for removal of toxic metal ion [30-32] due to presence of $–NH_2$ groups, which selectively binds with metal ions. However, at lower pH, $–NH_2$ groups undergo protonation therefore cannot bind with positively charged metal ions [33, 34].

Several other natural polysaccharides such as, sodium alginate [35], starch gaur gum, gum ghatti, okra, psyllium [36], are widely used for removal of toxic metal from aqueous solution. Polysaccharides have the various advantages over synthetic ones such as low cost, non-toxicity and biodegradability. The biodegradability is the demerit of natural polysaccharide because it reduces the self-life as well as the adsorption capacity [37]. The graft copolymerization is quite simple and very useful technique to modify the naturally occurring polysaccharide. The obtained grafted materials of polysaccharides are exhibiting the good the adsorption capacity.

5.2. Earlier method of synthesis

Singh et al. synthesized the psyllium and acrylic acid based polymeric hydrogel by using *N*,*N*-methylenebisacrylamide (*N*,*N*-MBAAm) as a crosslinker. The polymeric hydrogel was used for removal, separation, and enrichment of hazardous metal ions from aqueous solutions [38].

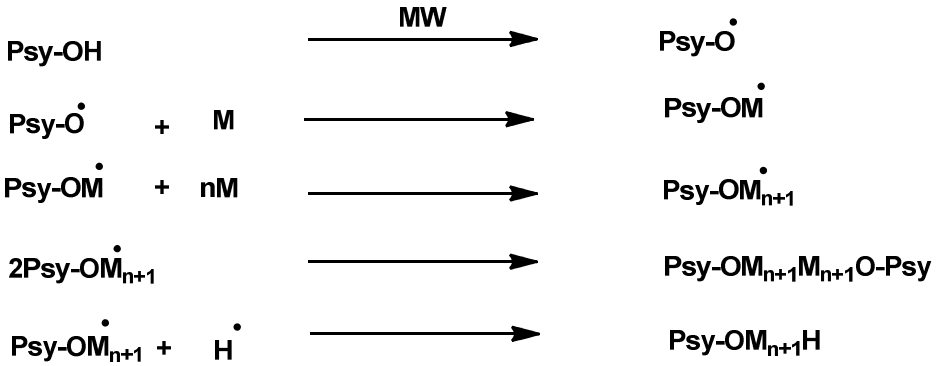
Rao et al. synthesized the hydrogel of psyllium and acrylic acid by using the *N,N*-methylenebisacrylamide as a crosslinker in presence of ammonium persulfate initiator. The crosslinked psyllium was characterized by PXRD, FTIR, DSC. They also studied the swelling behaviour of grafted psyllium and formed that swelling of crosslinked psyllium increased with decreased concentration of monomer and increasing concentration of crosslinker. (scheme 1) [39].



Scheme 1. Scheme of crosslinked hydrogel of psyllium.

Chaudhary et al. synthesized the Gum xanthan-psyllium-cl-poly (acrylic acid-co-itaconic acid) based adsorbent for effective removal of cationic and anionic dyes via microwave irradiation process. They achieved the excellent dye removal efficiency of 90.53% for EBT and 95.63% for Aur-O at initial dye concentration of 30 mg L^{-1} (EBT) and 15 mg L^{-1} (Aur-O) 40 mg L^{-1} with an adsorbent dose of 600 mg within time duration of 5 h at 323 K. The adsorption isotherm data fitted well with Langmuir isotherm and Freundlich isotherm for Aur-O and EBT dyes ($R^2 \geq 0.90$), respectively. The adsorption kinetics depicted that pseudo-second-order kinetics was followed simultaneously with intra-particle diffusion for both the dyes [40].

Sen et al. synthesized the polyacrylamide grafted psyllium (Psy-g-PAM) through microwave irradiation method without using any redox initiator as shown in scheme 2 [41].



Scheme 2. Scheme of Psy-g-PAM.

Dholakia et al. synthesized the acrylonitrile grafted sodium salt of partially carboxymethylated psyllium (Na-PCMPsy-g-PAN) using ceric ammonium nitrate (CAN) as a photoinitiator in an aqueous medium [42].

Jatav et al. synthesized the graft copolymer of psyllium with methacrylamide [psyllium-g-poly(methacrylamide)] through the microwave irradiation method. The grafted material was characterized by FTIR, TGA, XRD and SEM analysis and successfully used for the removal of metal ions, organic dyes from aqueous system and decoloration of dye solution [43]. Agarwal et al. prepared the psyllium grafted copolymer of *N*-vinyl 2-pyrollidone via microwave irradiation process and achieved the 195% grafting yield. The grafted sample was characterized by FTIR, SEM, XRD, TGA analysis technique [44].

5.3.Basis of work

Increasing water pollution is a very serious threat for the entire animal kingdom. All the animals, plants and human beings are directly or indirectly affected by discharging of industrial, domestic and medical wastes along with agricultural effluents into the rivers and groundwater, which disturb the biological balance of the aquatic system. Many natural polysaccharide adsorbent derivatives have been prepared for adsorbing metal ions and dyes from the water system. Keeping the above fact in mind and in continuation of our effort to develop the psyllium based best adsorbent for

wastewater treatment. we were interested in the synthesis of binary grafted psyllium for mercury removal from aqueous solution.

5.4. Present work

The present work deals with the synthesis and characterization of a binary grafted copolymer of psyllium with a mixture of acrylamide (Am) and acrylonitrile (An) for effective mercury removal application. The graft copolymers were synthesized *via* free radical polymerization using ceric ammonia nitrate/ascorbic acid [(CAN)/AA couple] as the free radical initiator under thermal conditions. The grafted compound was characterized via FTIR, SEM, XRD and thermal analysis. The Hg (II) adsorption capability of the adsorbent [Psy-g-Poly (Am-co-An)] through the batch adsorption method has also been studied.

5.5.Experiments

5.5.1. Materials

Psyllium husks were procured from Sidhpur Sat-Isabgol Factory India, acrylamide (Am), acrylonitrile (An), sodium hydroxide (NaOH), hydrochloric acid (HCl), methyl alcohol (MeOH), acetone (MeCoMe), gelatine, mercuric chloride ($HgCl_2$) were supplied by Merck Ltd. Mumbai, India. Rhodamine 6G and Potassium iodide (KI) were supplied by SD Fine chem. Ltd. Mumbai, India. Double distilled deionized water was used for synthesis as well as water analysis.

5.5.2. Synthesis of Psy-g-Poly (Am-co-An)

Grafted psyllium [Psy-g-Poly (Am-co-An)] was synthesized through our previously reported method [45]. Briefly, 1.0 g of psyllium mucilage (Psy) was dissolved in double distilled water in a two-necked round bottom flask. The required amount of acrylamide and acrylonitrile monomers were dissolved in distilled water (10 mL) in a conical flask and this solution was added to the psyllium solution. The round bottom flask wrapped by septum stopper assembly to flush nitrogen gas into the solution by using a hypodermic needle throughout the duration of the reaction. Later on an essential amount of CAN initiator was injected in the solution via hypodermic syringe and reaction mixture was continuously stirred at 30°C for a required time followed by adding 0.5 mL of saturated aqueous hydroquinone solution to terminate the

reaction [46, 47]. The reaction product [Psy-g-Poly (Am-co-An)] was allowed to precipitate out in methanol and washed with acetone and dried at 40°C.

The % grafting was calculated by the equation (1)-

$$\% \text{ Grafting } = \frac{\text{wt.of grafted polymer}}{\text{wt.of ungrafted polymer}} \text{x}100 \ldots \ldots \ldots \ldots (1)$$

5.5.3. Effect of various parameters variation onto the grafting

5.5.3.1.Monomers concentration

The effect of monomer concentration on to grafting was shown in Fig. 40 a and it was obtained from the binary mixture of both monomers (Am and An). The concentration of Am was varied from 0.07 to 0.28 mol/L in different sets of experiments while keeping the concentration of acrylonitrile (An) constant at 0.01 mol/L. The grafting increased with the increase in Am concentration (0.07 mol/L to 0.21mol/L), but as Am concentration was increased beyond 0.21mol/L, grafting started to decrease. The initial augmentation of monomers gradually increase the grafting with the dispersion of Am to the backbone psyllium as acrylonitrile was further increased, grafting decreased due to more homo polymerization [48, 49].

5.4.3.2. Initiator (CAN) concentration

The influence of couple initiator concentration (CAN) on % grafting is described in Fig. 40 b, where with increasing the concentration of initiator ($1.8\text{x}10^{-3}$ to $5.4 \text{ x } 10^{-3}$ mol/L), led to increase in the grafting yield due to the obtainability of more free radicals to initiate grafting at higher CAN concentration and further increase in the initiator concentration ($5.4\text{x}10^{-3}$ to $7.2\text{x}10^{-3}$ mol/L) decrease the grafting yield due to the favoured homo polymerization at high radical availability [50, 51].

5.4.3.3. Reaction time

The impact of time on % grafting is shown in Fig. 40 c. The grafting enhanced with increase in time from 30 to 124 minutes and then a slight decline in grafting percentage was observed. The quick increase of grafting between 60 and 102 minutes is due to the rate of initiation and propagation and the decline of grafting after 120 minutes is a clear remark of depletion of monomer concentration from the solution [46, 52].

5.4.3.4. Reaction temperature

The effect of reaction temperature on grafting parameters has been studied well in the temperature range of 20-50 °C as shown in Fig. 40 d**.** The enhancement in the reaction temperature (up to 50°C) increased the grafting. The increase in grafting yield with increase in temperature can be attributed to increased activity of initiator and monomers due to increase in the number of reactive sites [53].

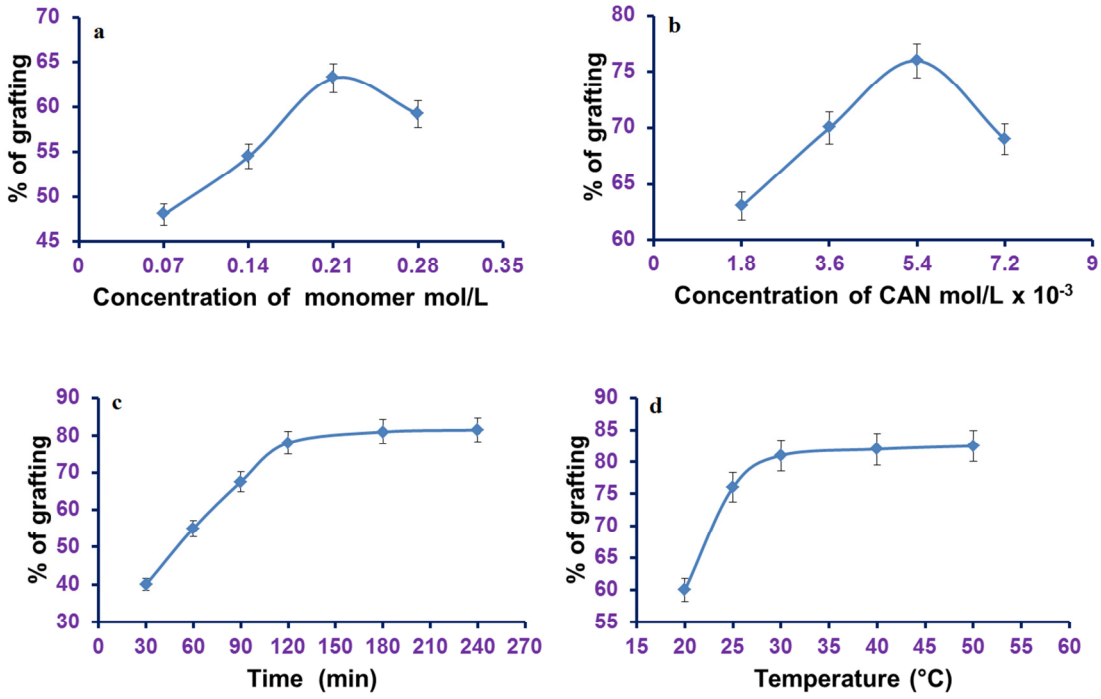


Fig.40. Effect of various parameters onto the grafting (a) Effect the monomer concentration on to grafting (b) Effect of initiator (CAN) Concentration on to grafting (c) Effect of reaction time on to grafting (d) Effect of reaction temperature on to grafting.

5.4.4. Characterization

5.4.4.1.FTIR spectra

The FTIR spectra of pure psyllium and Psy-cl-Poly (Am-co-An) are shown in Fig. 41a and 41b, respectively. The FTIR spectra of purified psyllium show a characteristic peak at 3392 cm^{-1}, due to stretching vibration of O-H whereas smaller peak at 2923 cm^{-1} is assigned to the C-H stretching vibrations. The peak at 1043 cm^{-1} is due to the C-O-C stretching vibrations. In case of Psy-cl-Poly (Am-co-An), peaks at

2240.78 cm^{-1} (C≡N [54] nitrile stretching), 1726, 1673.25 cm^{-1} (C=O stretching of amide-I) [55], 1423 cm^{-1} (N-H in-plane bending of amide-II) and 1251 cm^{-1} (C-N stretching of amide-III) were also observed in addition to the peaks observed with IR of pure psyllium.

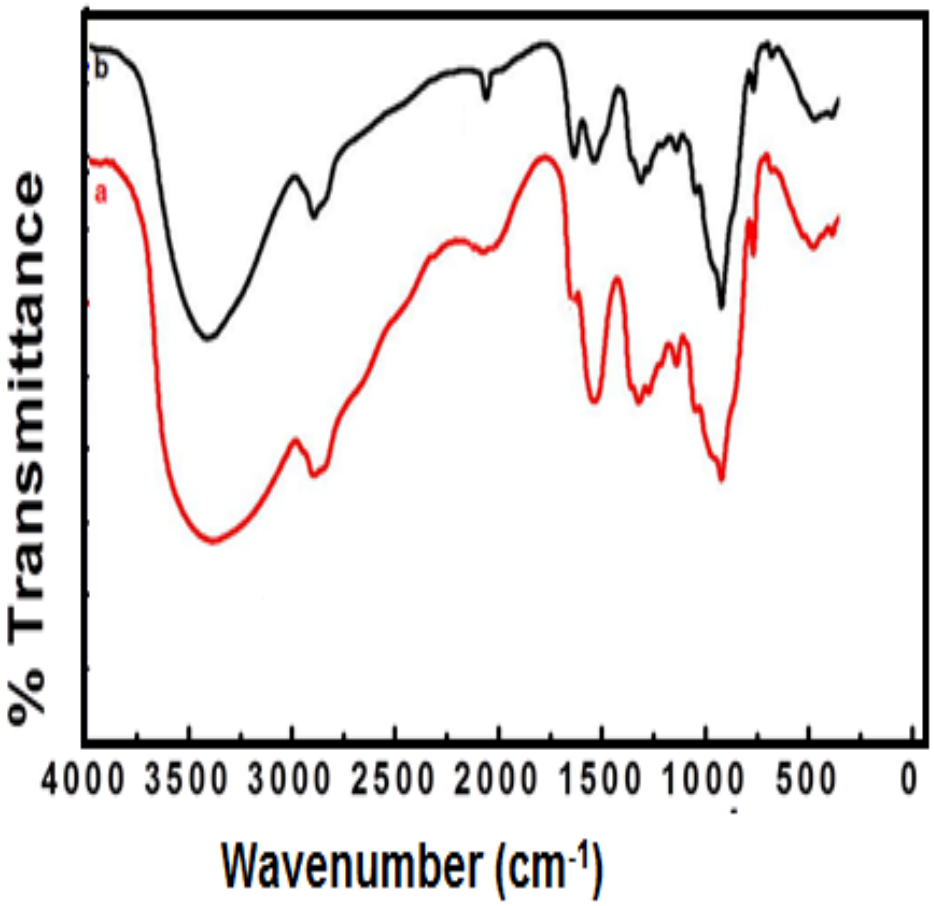


Fig. 41. FT-IR spectra of the (a) Psyllium, (b) Psy –g- Poly (Am-co-An).

5.4.4.2.Scanning electron microscopy

The surface morphology of pure psyllium and binary grafted psyllium [Psy-g-Poly (Am-co-An)] has been studied by SEM and the surface morphology of psyllium and Psy-g-Poly (Am-co-An) are shown in Fig. 42. It has been observed from the SEM that psyllium has smooth and homogeneous surface morphology whereas modified psyllium has roughly and structural heterogeneity. The homogeneous surface of pure psyllium was vanished after grafting and converted into heterogeneous morphology.

5.4.4.3.XRD analysis

The XRD investigation of any material provides evidence about its crystalline nature. XRD patterns of Psyllium and Psy-g-Poly (Am-co-An) are shown in Fig. 43a and (43b), respectively. The XRD patterns of psyllium show a broad a peak at 2θ = 22°, indicating amorphous nature [56, 57]. Whereas, in case of Psy-g-Poly (Am-co-An) one peaks at 2θ = 22°, also describes amorphicity. On investigating both XRD spectra it was found that the diffraction [58] peak intensity of psyllium are not significantly decreased after grafting at all angles but the XRD of grafted spectrum present slightly broad spectrum as compared to pure psyllium means slightly increase the amorphous nature of psyllium.

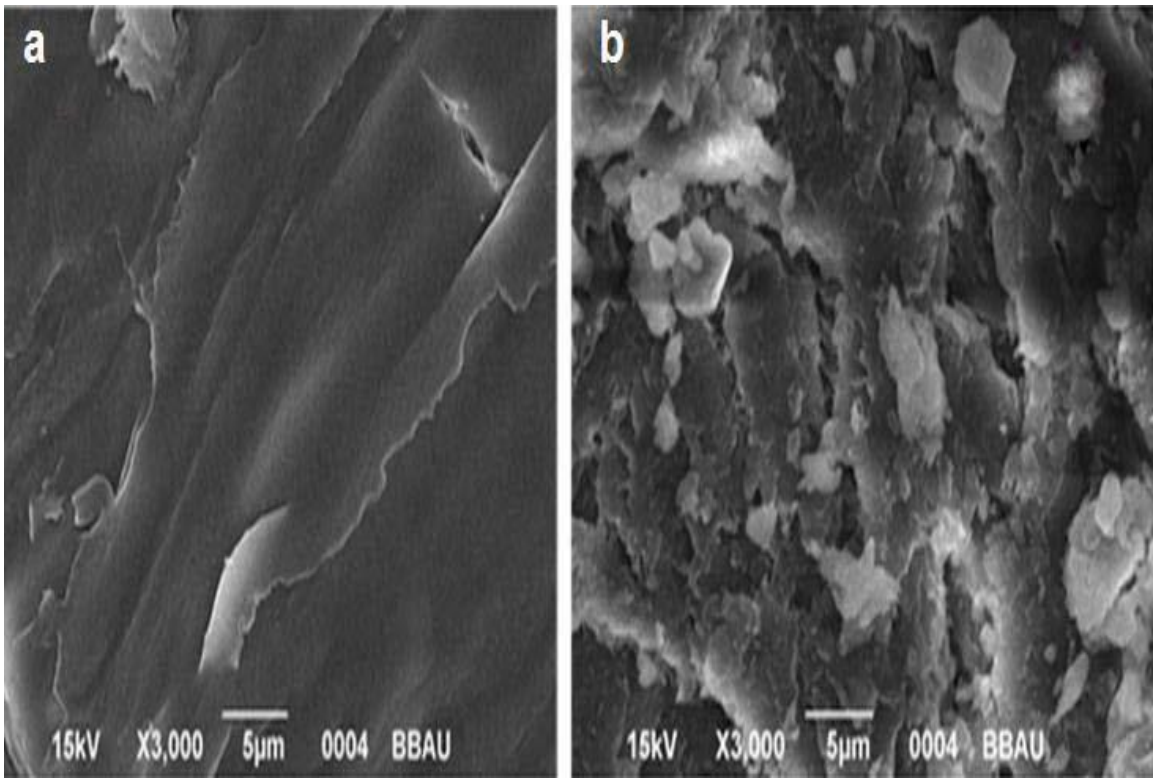


Fig. 42. SEM images of the (a) Psyllium (b) Psy-g-Poly (Am-co-An).

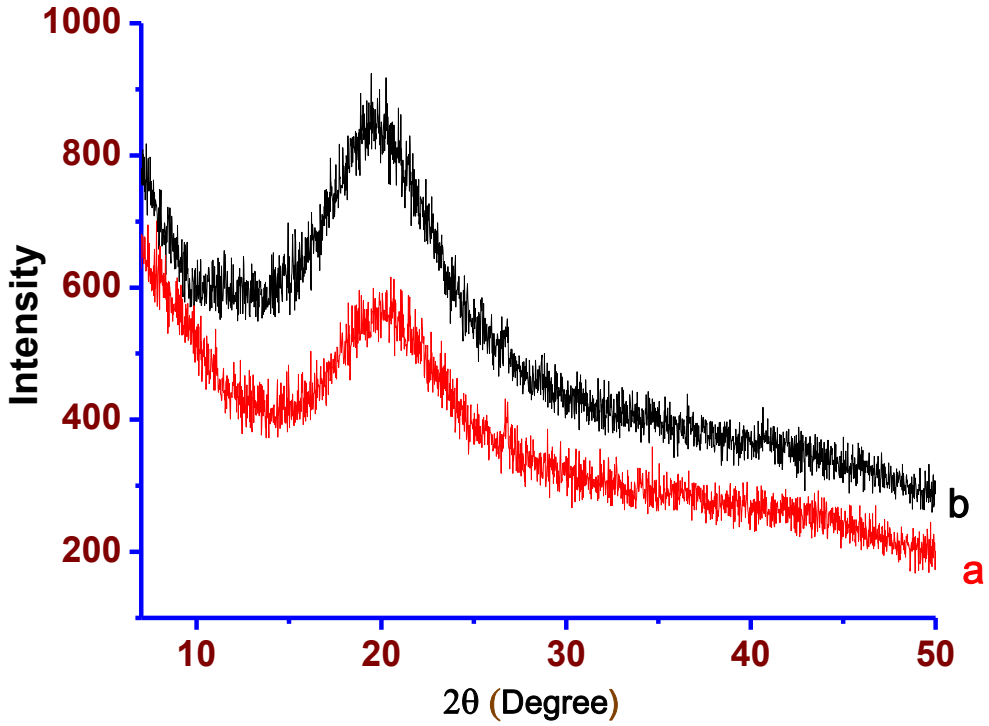


Fig. 43. XRD spectra of the (a) Psyllium, (b) Psy-g-Poly(Am-co-An).

5.4.4.4. Thermal behavior

Thermal study (TGA/ DTA/DTG) of pure psyllium and Psy-g-Poly (Am-co-An) were displayed in Fig. 44. TGA curves of psyllium and Psy-g-Poly (Am-co-An) were achieved by scanning both the samples from 0 °C to 800 °C. We previously reported that TGA curves of psyllium (Fig. 44a) show two weight loss step [45]. Initial weight loss is about 12.2 % between temperatures 30-250°C which is due to the traces of moisture present. Again weight loss of 83.8 % is detected near 442°C which is due to breakage of psyllium backbone. Psy-g-Poly (Am-co-An) is also showing two thermal degradation stages. The first stage ranged from 25°C to 250°C (14.5%) corresponding to the removal of water. In the second stage, the weight loss is 79.3% in the temperate range of 350-500°C. Decomposition of Psy-g-Poly (Am-co-An) started at 100°C and 50% weight loss of Psy-g-Poly (Am-co-An) was occurred upto the temperature 350 °C. Finally, the polymer backbone of Psy-g-Poly (Am-co-An) was completely degraded at 500 °C as shown in Fig. 44b, which is slightly higher as compared to original psyllium

(Table-8) [59]. DTA and DTG curve, also show that grafted psyllium is comparatively more stable as compared to pure psyllium.

Table 8:- Thermal analysis of Psyllium and Psy-g-Poly (Am-co-An) samples.

Pure Psyllium		**Psy-g-Poly (AA-co-An)**	
Temperature (^{o}C)	**% weight loss**	**Temperature (^{o}C)**	**% weight loss**
100	5.97	100	8.45
200	10.4	200	12.44
250	12.2	250	14
300	57.36	300	48.8
400	78.9	400	68.1
442	96	442	-
500	-	500	96

5.4.4.6.Zeta potential

To investigate and determine the surface properties of newly modified psyllium were used the zeta potential was measured. Zeta potential of Psy-g-Poly (Am-co-An) was observed negative with less mobility as given in Table -9**,** which clearly indicates their considerable stability in the mercury removal from its solution (Fig. 45).

Table 9:- - Measurement of Zeta potential.

Zeta potential	: -16.8 (mV)	Doppler Shift	: 15 (Hz)
Mobility	: -2.0-4 (cm^2/Vs)	Base frequency	: 123 (Hz)
Conductivity	: 0.133 (mS/cm)	Conversion Equation	: smoluchowski

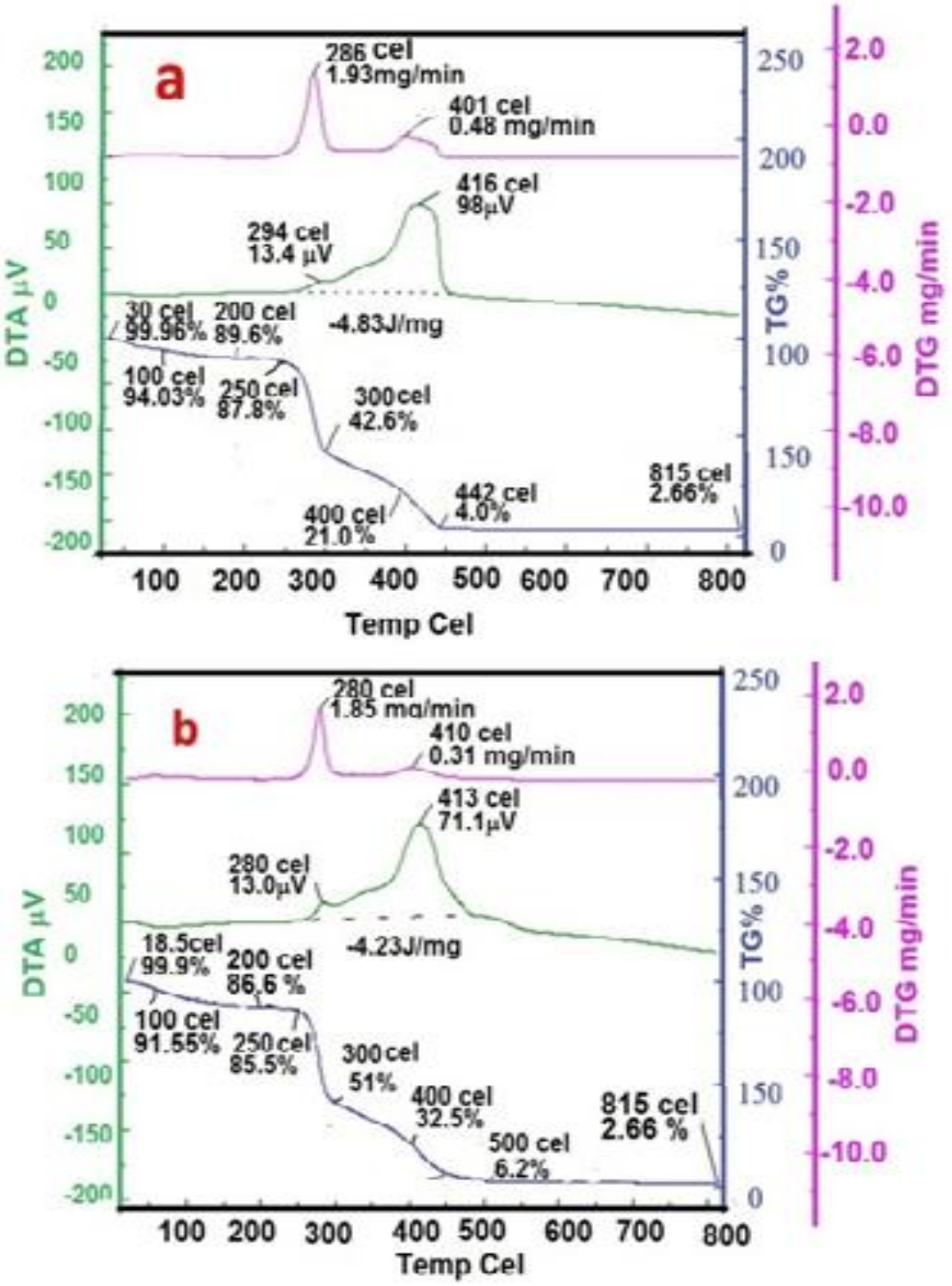


Fig. 44. TGA, DTA and DTG curves of (a) Psyllium (b) Psy-g-poly (Am-co-An).

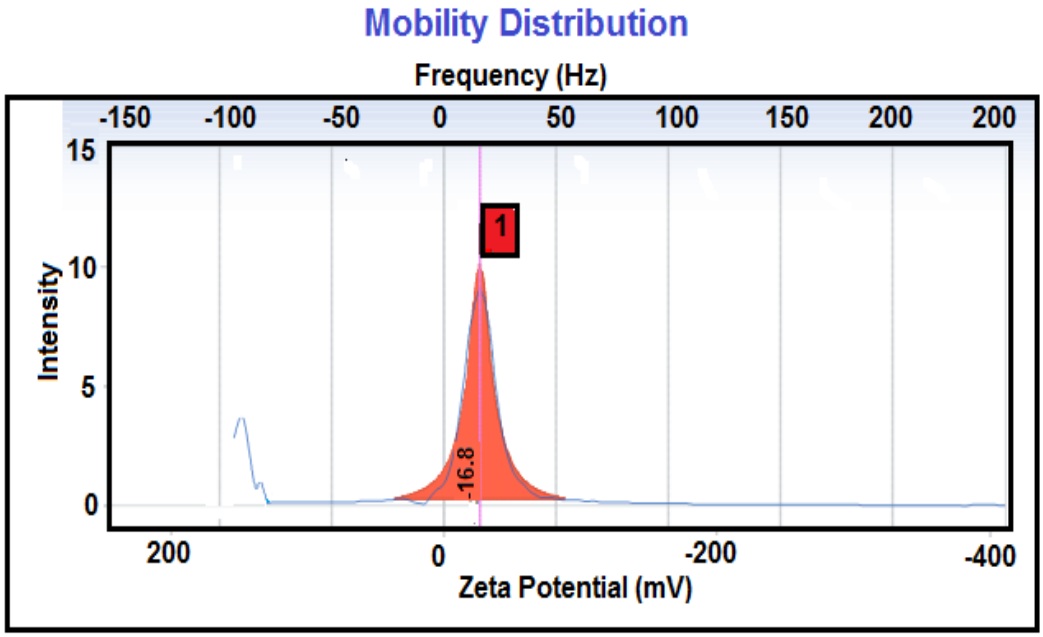


Fig. 45. Zeta potential.

5.4.5. Hg (II) adsorption method

A standard solution of 1000 ppm of Hg (II) was obtained by dissolving 1.354 g of $HgCl_2$ in 1L deionized double distilled water [60]. All mercury (II) adsorption experiments [61] were investigated at normal temperature. The impact of various influences like adsorbent amount, contact time, pH, Hg (II) concentration etc. was investigated by batch adsorption experiment. The impact of pH on mercury (II) adsorption was investigated at various pH by adjusting pH with 0.1 M HCl or 0.1 M NaOH [62]. 20 mL Hg (II) solution (100 ppm) was taken in 50 mL beaker added 20 mg adsorbent was added and stirred with magnetic stirrer for the desired time period, and filtered the solution using Whatman 0.45mm filter paper. After appropriate dilution, the remaining quantity of Hg (II) was measured by a double beam UV spectrophotometer (λ-575nm) using the rhodamine 6G dye and iodine buffer solution [63, 64]. The quantity of Hg^{2+} adsorbed by grafted copolymer in ppm was calculated by [65] following equation (2),

$$q_e = \frac{Q_e - Q_o}{W} \text{ xV} \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Where q_e = the amount of the metal adsorbed (ppm) onto the adsorbent, Q_o=the initial concentration of solution (ppm), Q_e=equilibrium concentration of solution (ppm), V=volume and W= adsorbent weight.

The adsorption of Hg (II) by Psy-g-Poly (Am-co-An) has been investigated by varying only one adsorption parameter at a time while others kept constant. Various adsorption parameters and their range [pH (4 to 10), adsorbent dose (10 mg-70 mg), temperature (15°C to 50°C), contact time 60 minutes and contact volume 10 mL at 100 ppm mercury(II) concentration] were studied.

5.4.5.1. Effect of various parameters onto the adsorption

5.4.5.1.1. Adsorbent dose

The impact of adsorbent [(Psy-g-Poly (Am-Co-An)] dose on Hg (II) adsorption was studied from 10 to 50 mg, keeping other parameters constant which affect the adsorption and the outcomes are mentioned in Fig. 46a. It was observed that removal of Hg (II) increased from 56.5% to 89.9% with rise in the adsorbent dose from 10 mg to 30 mg due to the availability of more binding sites at higher doses and further increase in adsorbent dose from 30 to 50 mg result in nominal increase in elimination of Hg (II). Therefore, 30 mg adsorbent dose was selected designated for further optimization and kinetic studies.

5.4.5.1.2. Influence of pH

The impact of pH on the Hg (II) removal was investigated in the pH range 2-8 keeping other parameters constant which affect the adsorption. The outcome is given in Fig. 46 b. It was observed that the percentage removal of Hg (II) increases from 41% to 92% with rise in pH from 2 to 6, because at low pH mercury exists as Hg^{2+} whereas on further increase of pH from 7 to 8, decrease the percentage elimination of Hg(II) due the formation of $Hg(OH)_2$ [66] and therefore, pH 6 was selected for kinetic studies, as at pH 6 mercury exists as positive ion (Hg^{+}) [38].

5.4.5.1.3. Contact time

The study of the removal of Hg (II) was performed with a fixed adsorbent dose at several time intervals (10-120 minutes). The result is shown in Fig. 46c. It was investigated that the percentage removal [67] of Hg (II) increases from 53.5% to 94.9% with the increase in adsorption time from 10 to 60 min due to increase in the metal binding time with vacant

adsorbing sites. Further increase in the time beyond 60 min did not lead to any significant increase in the adsorption due to the optimum capacity of adsorption sites [11].

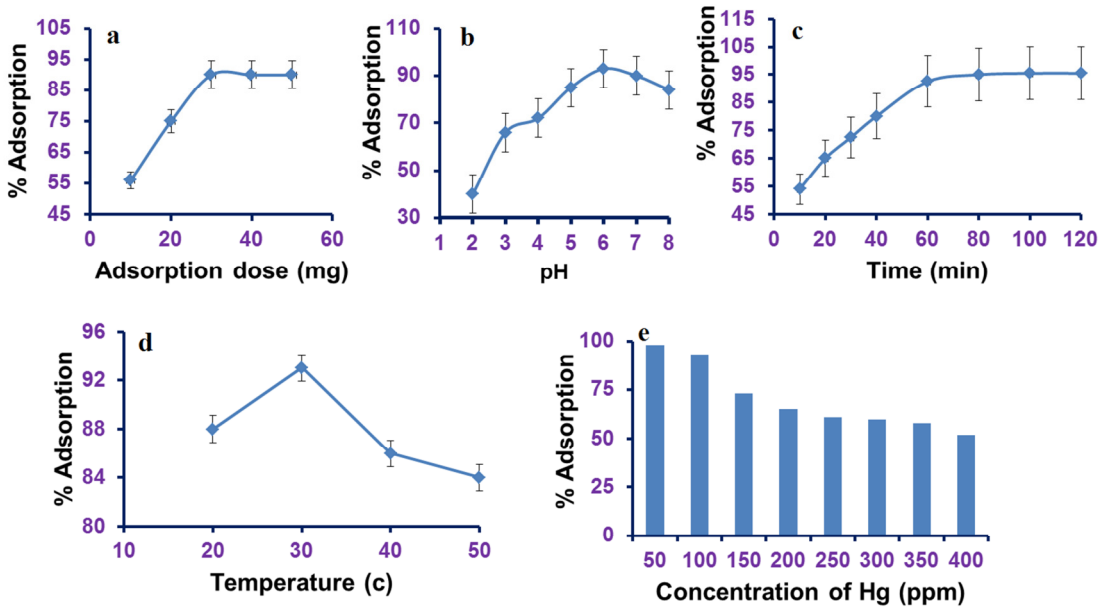


Fig. 46. Effect of various parameters onto the Hg adsorption (a) Effect of adsorbent dose on to Hg sorption (b) Effect of pH variation on to Hg sorption(c) Effect of time variation on to Hg sorption (d) Effect of temperature variation on to Hg sorption (e) Effect of initial Hg (II) ion concentration on to Hg sorption.

5.4.5.1.4. Temperature

The impact of temperature on the Hg (II) adsorption was performed in the range of 20–50 °C under constant parameters are shown in Fig. 46 d. The mercury adsorption continuously increased with the increase in the temperature from 20-30 °C; due to increase in the active surface centre site for sorption. Further increase in the temperature, decrease the adsorption due to some desorption phenomenon taking place above 30 °C.

5.4.5.1.5. Initial Hg (II) ion concentration

Effect of initial concentration of Hg (II) ion on adsorption when initial concentration of Hg (II) ion was varied from 50 to 400 ppm at a particular time, particular pH and temperature was displayed in Fig. 46 e. With the increase in the initial concentration of Hg (II) from 50 to 400 ppm, firstly (50 to 200ppm) mercury adsorption quickly increases due to

the availability of extra mercury (II) ions for the binding. After that mercury adsorption slowly increases.

5.4.6. Adsorption Isotherm Studies

For isotherm investigation, the adsorption equilibrium [68] data were originated at various initial Hg (II) concentrations ranging from 50 ppm interact time at room temperature. Various adsorption models give the information that how particles subjected to adsorption distribute themselves between adsorbate and adsorbent phases at equilibrium time [69]. They offer some insight into the adsorption mechanism, surface properties and affinities of the adsorbent. In this study, the Langmuir model (monolayer adsorption) and Freundlich model (multilayer adsorption) are used. The equilibrium sorption of the Hg^{2+} ions was carried out by 30 mg of the Psy-g-Poly (Am-co-An) with 20 mL of 100 ppm of different concentrations from 50 to 400 ppm in 25 mL conical flasks for 60 minutes on the shaker and mixture was filtered [70] and filtrate was used to test the metal ion concentration through UV spectrophotometer. Data were fitted into the Langmuir and Freundlich adsorption isotherm [70, 71].

5.4.6.1. Langmuir Adsorption Isotherm

Langmuir adsorption [72] isotherm is highly effective for monolayer sorption because the surface has finite number of identical sites and expressed in the linear form as equation (3).

$$\frac{C_e}{Q_e} = \frac{K_L}{Q_m} + \frac{C_e}{Q_m} \dots\dots\dots\dots\dots\dots (3)$$

Where

C_e = Equilibrium concentration

Q_e = Amount adsorbed at equilibrium

Q_m = Langmuir constants

K_L = Heat of adsorption.

The vital characteristics of Langmuir model are explained through means of R_L (dimensionless constant) and R_L is calculated from the equation (4).

$$R_L = \frac{1}{(1 + K_L\ \mathrm{Co})} \dots\dots\dots\dots (4)$$

Where

C_0 = Hg (II) concentration (mg/L).

Adsorption is favorable when the R_L value is between 0 and 1.

The value of Q_m (57.47 mg/g) was calculated from Langmuir model, indicating that the adsorbent showed the high capacity to remove mercuric ions (Fig. 47 a). R_L and K_L were determined to be 0.01493 and 0.01649 ml/mg respectively, thus adsorption is favorable.

5.4.6.2. Freundlich Adsorption Isotherm

Freundlich isotherm defines the heterogeneous [73] surface energy through multilayer adsorption [72, 73] and indicates the linear form as equation (5).

$$\ln q_e = \ln K_f + n \ln Ce \ldots\ldots\ldots\ldots\ldots (5)$$

Where

K_f = Adsorption capacity of adsorbent

Value of Freundlich parameters (K_f), correlation constant (R^2) and rate constant were calculated by Freundlich isotherm (Fig. 47 b) given in Table-10. The equilibrium data fitted to Langmuir (R^2 = 0.9976) model better than Freundlich model (R^2 = 0.9434) indicating surface homogeneity of adsorbent and monolayer adsorption.

Table 10:- Correlation coefficients and constant parameters calculated for Langmuir Adsorption Isotherm and Freundlich adsorption models for Hg (II).

Langmuir Adsorption Isotherm	**Freundlich Adsorption Isotherm**
Q_m = 1/ slop = 57.47	Kf = Antilog (intercept) = 3.432
Kl = 1/(Q_m x intercept) =0.1649	n = 1/ slip = 4.0883
Rl = 1/(1+Kl x Ce)= (0.1081-0.0149)	R^2 = 0.9434
R^2 = 0.9976	-

5.4.7. Kinetic Studies

In order to investigate [74] kinetic data, the interaction time was varied from 10 to 120 minutes and the kinetic studies were completed by using 100 ppm Hg(II) concentration [75], 30 mg adsorbent dose at pH 6 and temperature 25 °C.

The rate of mercuric [76] ions removal from the aquatic system by the adsorbent in significant [2] equilibrium time, effect of interaction time for removal of Hg (II) by using many initial mercuric ions concentrations was demonstrated in Fig. 48.

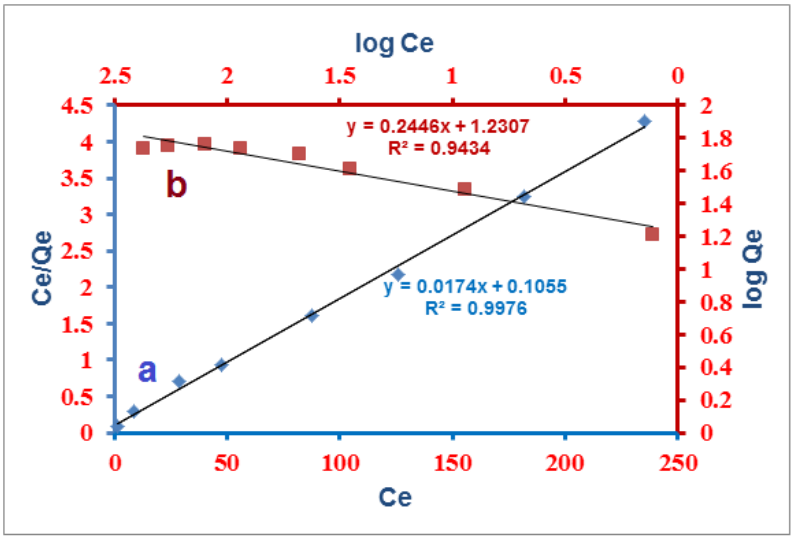


Fig. 47 (a) Langmuir (b) Freundlich adsorption model for the adsorption of Hg.

It is observed that the adsorption reaction was initially fast, and at equilibrium time, become slow [77] because a great number of unoccupied surface sites are presented for adsorption in initial phase. Six of the most widely used kinetics models: first order [78], Elovich equation [79], second order [80], pseudo second-order [2], intra-particle diffusion [81] and pseudo-first [82] order models were used to evaluate kinetic mechanism of the Hg (II) adsorption onto Psy-g- Poly (Am-co-An).

5.4.7.1. First -order kinetics equation

The linear form of first-order kinetics equation is given as equation (6).

$$\ln\frac{Q_o}{Q_t} = k_1 t \text{.....(6)}$$

Where Q_o (mgL^{-1}) and Q_t (mgL^{-1}) are concentration at the time zero (initial) and a given time‘t’ concentration of metal ions in solution respectively. K_1 (min^{-1}) is the first order [78, 83] rate constant and regression R^2 obtained by the linear plot of ln (Q_o/Q_t) vs t (Fig. 48 a), is shown in Table-11. For mercury, R^2 was more than 0.9, which shows a good fit the experimental data.

5.4.7.2. Second order rate equation

The linear form second-order kinetics equation is given in equation (7) below:

$$\frac{1}{(Q_o - Q_t)} = k_2 t \ldots\ldots\ldots\ldots (7)$$

Where K_2 [$Lmg^{-1}min^{-1}$] is the second order [84] rate constant for the sorption process, determined from the linear plot of ($1/Q_t - 1/Q_o$) against t, shown in Fig. 48b for mercury refer the Table- 5.4 above for the value of the constants.

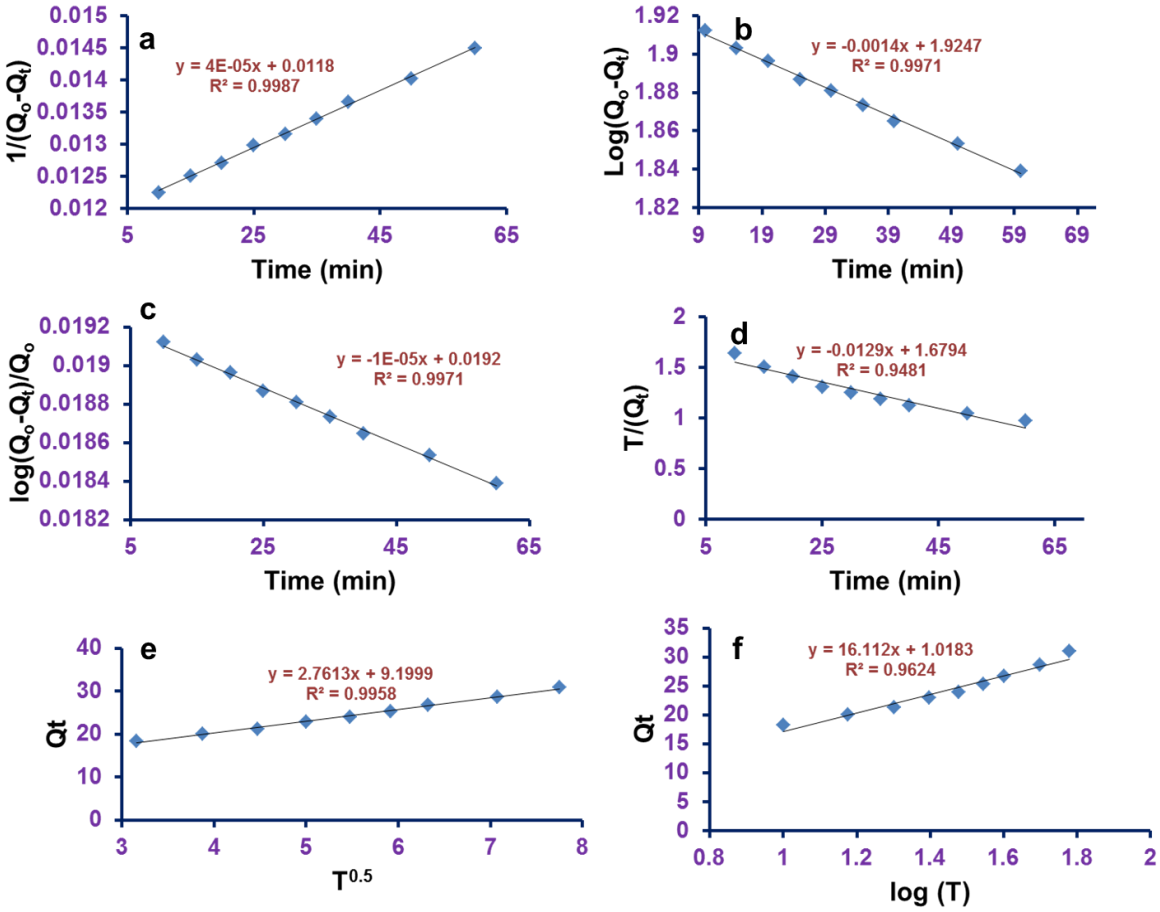


Fig. 48. Kinetic models for the adsorption of Hg(II) by Psy-g-poly(Am-co-An) (a) first order (b) second order (c) pseudo-first order (d) pseudo-second-order (e) intra-particle diffusion (f) Elovich model.

5.4.7.3. Pseudo-first-order kinetic equation

Linear form pseudo [85] first order equation [9] is given in equation (8).

$$\log\frac{(Q_o - Q_t)}{Q_o} = \log Q_o - \frac{k_1 t}{2.303} \ldots\ldots\ldots\ldots (8)$$

Where, Q_t, Q_0 and k_1 are adsorbate at time t, adsorption ability [9, 15] at equilibrium, and rate constant respectively. All parameters of this equation were calculated via Fig. 48 c (result is shown in Table-11).

5.4.7.4. pseudo-second order kinetics equation

The pseudo-second [2] order kinetic rate was studied by equation (9) [66].

$$\frac{t}{Q_t} = \frac{t}{k_2 Q_e^{\,2}} + \frac{t}{Q_e} \ldots\ldots\ldots\ldots (9)$$

Where k_2 represents rate constant. The plot for the equation (9) was demonstrated in Fig. 48 d which shows the data was perfectly fitted to the model and value of all parameters were given in Table-11. R^2 is 0.99 which indicated that the adsorption system was highly in accordance with this [86] kinetic mechanism compare to other kinetic mechanisms. Therefore, it supports the assumption behind the model and suggests that the overall rate of Hg (II) adsorption by psy-g-Poly (Am-co-An) appeared to be controlled by the physicochemical process.

5.4.7.5. Intraparticle diffusion

Equation (10) is Intraparticle diffusion [87] kinetic equation.

$$Q_t = K_{id}\, t^{0.5} + C \ldots\ldots\ldots (10)$$

Where K_{id} is rate constant of intraparticle diffusion (mg g^{-1} $min^{-0.5}$) and C is intraparticle diffusion constant (mg g^{-1}). Fig. 48 e shows the intraparticle diffusion kinetic curve for mercury adsorption and the calculated value of intraparticle diffusion parameters were given in Table-11. It was observed that the rate constant enhanced with enhanced mercury concentration.

5.4.7.6. Elovich rate equation

The Elovich equation [79] is given in equation (11)

$$Q_t = \alpha \log a\alpha + \alpha \ln t \ldots\ldots\ldots (11)$$

Where Q is the amount adsorbed at t time and α (g/mg) and a (mg/$g^{-1}min^{-1}$) are the Elovich constants. these constants can be observed as the initial rate since dQ/dt → as Q =0 [88]. These values of α and a were calculated with the help of linear plot of Q_t Vs lnt (Fig. 48 f) and presented in Table-11.

Table 11:- Comparison of first order, second order, pseudo-first order, pseudo-second-order, intra-particle diffusion and Elovich equation models parameters for the sorption by Psy-g-

poly (Am-co-An) at initial Hg (II) concentration of 100 mg L^{-1}, dose 30 mg, contact volume 10 mL and temperature 25 ^{o}C.

S.N.	Kinetic model	Linear form	Plot	Parameter
1	First-order rate equation	$\ln\frac{Qo}{Qt} = k1t$	$\ln\frac{Qo}{Qt}$ Vs t	K1=0.0014 R^2= 0.9971
2	Second order rate equation	$\frac{1}{(Qo-Qt)} = k2t$	$\frac{1}{(Qo-Qt)}$ Vs t	K2=4x10^{-5} R^2=0.9987
3	Pseudo first-order equation	$\log(Qo-Qt)/Qo = \log Qo - \frac{k1t}{2.303}$	$\log\frac{(Qo-Qt)}{Qo}$ Vs t	K1= 2.3x10^{-5} R^2=0.9971
4	Pseudo-second-order rate equation	$\frac{t}{Qt} = \frac{t}{k_2Qe^2} + \frac{t}{Qe}$	$\frac{t}{Qt}$ Vs t	Qo=77.51 K2= 9.9x10^{-5} R^2=0.9481
5	Intraparticle diffusion	$Qt = kid\ t^{.5} + C$	Qt Vs $t^{.5}$	Kid=2.76 C= 9.16 R^2=0.9958
6	Elovich equation model	$Qt = \alpha\log(a\alpha) + \alpha\ln(t)$	Qt Vs ln(t)	α = 16.11 a=0.982 R^2=0.9624

5.5. Conclusion

The Psy-g-poly (Am-co-An) graft copolymer adsorbent was successfully synthesized and characterized using many techniques and has been optimized by changing the various

reaction parameters, monomers concentration (acrylamide and acrylonitrile), temperature, reaction time and initiator concentration. We have achieved the maximum grafting yield at 0.21 mol/L Am monomer concentration (An fixed at 0.01 mol/L), $5.4x10^{-3}$ mol/L CAN/AA concentration, 180 minutes and 40 °C temperature. Psy-g-poly (Am-co-An) proved to be a highly efficient mercury ion sorbent. The adsorption of mercuric ions through Psy-g-poly (Am-co-An) was found to be pH dependent and pH 6 was found to be highly suitable for the sorption. The adsorption followed kinetic of the second order, which indicated chemisorption mechanism and adsorption isotherm was analyzed using the Langmuir and Freundlich isotherms and found that adsorption isotherm follows the Langmuir model indicating unilayer sorption.

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List of Publications

- **Deepak Kumar,** Jyoti Pandey, Pramendra Kumar, Microwave assisted synthesis of binary grafted psyllium and its utility in anticancer formulation, **Carbohydrate Polymers** 179 (2018) 408–414. (**I.F. 5.1**) **(Elsevier)** (UGC J. No- 5035)
- **Deepak Kumar,** Jyoti Pandey, Pramendra Kumar, Binary grafted chitosan film: Synthesis, characterization, antibacterial activity and prospects for food packaging **International Journal of Biological Macromolecules,** 115, (2018), Pages 341-348. (**I.F. 3.909**) **(Elsevier)** (UGC J. No- 2634)
- **Deepak Kumar,** Jyoti Pandey, Pramendra Kumar, Synthesis and characterization of modified chitosan via microwave route for novel antibacterial application, **International Journal of Biological Macromolecules,** 107 (2018) 1388–1394. (**I.F. 3.909**) **(Elsevier)** (UGC J. No- 2634)
- **Deepak kumar,** Pramendra kumar, Vinit Raj, Jyoti Pandey, A Review on the Modification of Polysaccharide Through Graft Copolymerization for Various Potential Applications **The Open Medicinal Chemistry Journal, (**2017), 11, 109-121. (**I.F. 1.27**) **(Bentham Science)** (UGC J. No- 36404)
- **Deepak kumar,** Pramendra kumar, Nida Khan, Jyoti Pandey, "Improve the native characteristics of polysaccharides by grafting through the gamma radiation: A Review" **Green Chemistry & Technology Letters,** 3, (2016), 151-159. (UGC J. No-46979)

List of Conferences and Workshops

- Three days international conference in Department of Physics on topic **"Emerging Materials and Applications**" organised by University of Allahabad, Allahabad, Uttar Pradesh, India **(Poster Presentation).**
- Four days international conference in Department of Applied Physics on topic **"New Scintillation on Materials Horizon**" organised by M.J.P. Rohilkhand University Bareilly, Uttar Pradesh, India **(Oral Presentation).**
- Two days international conference in Department of Biotechnology Science on topic **"New Frontiers in Biotechnology Science, Health & Medicine**" organised by Invertis University Bareilly, Uttar Pradesh, India **(Poster Presentation).**

- Two days national conference in Department of Chemistry on topic **"Brass Metal Works, Health and Environment**" organised by Moradabad Muslim Degree College Moradabad Uttar Pradesh, India **(Oral Presentation).**
- Four days national conference on topic **"3rd Lucknow Science Congress**" organised by Babasaheb Bhimrao Ambedkar University, Lucknow Uttar Pradesh, India **(Oral Presentation).**
- Two days national conference in topic **"Impact of Rapid Advancement in Science and Technology**" organised by Rajshree Institute of Management & Technology, Bareilly, Uttar Pradesh, India **(Oral Presentation).**
- Five days national workshop in Department of Applied Chemistry on topic **"Innovation Research on Material in Science and Engineering**" organized by M.J.P. Rohilkhand University Bareilly, Uttar Pradesh, India **(Participated).**
- Three days national norkshop on title **"Hands - on – Training on SEM, FTIR, FPLC and Ion Chromatography**" organised by Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India **(Participated).**

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