SYNTHESIS OF CHITOSAN POLYVINYL ALCOHOL METHIONINE HYDROGEL AND ITS SWELLING RESPONSES IN PHYSIOLOGICAL FLUIDS

SIVAGAMI R M

20PCH020

Dissertation work submitted to
Avinashilingam Institute for Home Science and Higher
Education for Women, Coimbatore – 641043,
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In Partial fulfilment of the Requirement for the Degree of
MASTER OF SCIENCE IN CHEMISTRY
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26/5/2022
Signature of the
Supervisor

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Signature of the
Head of the Department

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Sivagami R M

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LIST OF ABBREVIATIONS

- **SAP** - Super Absorbent Polymer
- **SPH** - Super Porous Hydrogels
- **CS** - Chitosan
- **CSPVAM** - Chitosan polyvinyl alcohol Methionine
- **PVA** - Polyvinyl Alcohol
- **PVAM** - Polyvinyl Alcohol Methionine
- **MBA** - N, N’ – methylene bisacrylamide
- **IPN** - Semi-Interpenetrating Polymer Network
- **EC** - Ethyl Cellulose
- **CMC** - Carboxymethyl cellulose
- **PAAm** - Polyacrylamide
- **AAm** - Acrylamide
- **pNIPAAm** - Poly (N-isopropylacrylamide)
- **PEG** - Polyethylene glycol
- **PGA** - Polyvinyl alcohol/Glycerol/Polyaniline
- **PHEMA** - Poly-2-hydroxyethyl methacrylate
- **PNIPAAm** - Poly-N-isopropylacrylamine
- **PANI** - Polyaniline
- **PLA** - Polylactic acid
- **PAM-PVA** - Polyacrylamide – Polyvinyl alcohol
- **PHA** - Polyhydroxyalkanoates
- **CPs** - Conducting Polymer
- **CPHs** - Conducting Polymer Hydrogels
- **PVP** - Polyvinylpyrrolidone
- **NaOH** - Sodium Hydroxide
- **NaCl** - Sodium Chloride
- **$MgSO_4$** - Magnisium Sulphate

- **$CaCl_2$** - Calcium Chloride
- **KI** - Potassium Iodide
- **KBr** - Potassium Bromide
- **KCl** - Potassium Chloride
- **HCl** - Hydrochloric Acid
- **S.W** - Saline Water
- **S.U** - Synthetic Urine
- **LCST** - Lower Critical Solution Temperature
- **WRC** - Water retention capacity
- **AUL** - Absorbency under load
- **SPR** - Surface plasmon resonance
- **FTIR** - Fourier Transform Infrared Spectroscopy
- **PXRD** - Powder X- Ray Diffraction
- **TG** - Thermogravimetry Analysis
- **DTA** - Differential Thermal Analysis
- **DTG** - Differential Thermogravimetry Analysis

1. INTRODUCTION

Hydrogels are three dimensional dimensional networks of hydrophilic polymers, possessing a margin amount of water. Hydrogel has a tunable properties and versatile fabrication method. These networks establish equilibrium with the liquid and temperature of their surroundings for shape and mechanical strength. Early work on hydrogels was initiated in the mid 1930's with the studies on kinetics of cross-linked polymers. Hydrogels undergo a significant volume phase transition or gel-sol phase transition in response to certain physical and chemical stimuli. The physical stimuli include temperature, electric and magnetic fields, solvent composition, light intensity, and pressure, while the chemical or biochemical stimuli include pH, ions, and specific chemical compositions. However, in most cases such conformational transitions are reversible; therefore, the hydrogels are capable of returning to their initial state after a reaction as soon as the trigger is removed. The response of hydrogels to external stimuli is mainly determined by the nature of the monomer, charge density, pendant chains, and the degree of cross-linkage. The magnitude of response is also directly proportional to the applied external stimulus. (**Morteza Bahram, et. al, 2016)** in their article reviewed that based on the source hydrogels can be classified as Natural hydrogels, Synthetic hydrogels. Natural hydrogels include collagen, fibrin, hyaluronic acid, matrigel, and derivatives of natural materials such as chitosan, alginate and skill fibers. They remain the most physiological hydrogels and synthetic hydrogels such as poly (ethylene glycol) diacrylate, poly (acryl amide), poly (vinyl alcohol) etc. Synthetic hydrogels offer more flexibility for tuning chemical composition and mechanical properties.

1.1 Classifications of hydrogels

According to the source, hydrogels can be divided into those formed from natural polymers and those formed from synthetic polymers. Depending on the ionic charges on the bound groups, hydrogels may be cationic, anionic, or neutral. The types of cross-linking agents also can be the criteria for classification. Hydrogels can be physical, chemical, or biochemical. (**Eleonora Parelius Jonasova, et. al, 2016).** Physical gels can undergo a transition from liquid to a gel in response to a change in environmental conditions such as temperature, ionic concentration, pH, or other conditions such as mixing of two components. Chemical gels use covalent bonding that introduces mechanical integrity and degradation resistance compared to other weak materials. In biochemical hydrogels, biological agents like enzymes or amino acids participate in the gelation process. It is also possible to divide hydrogels into groups based on their structure: amorphous, semicrystalline, crystalline, and hydrocolloid aggregates.**(Morteza Bahram, et. al, 2016).**

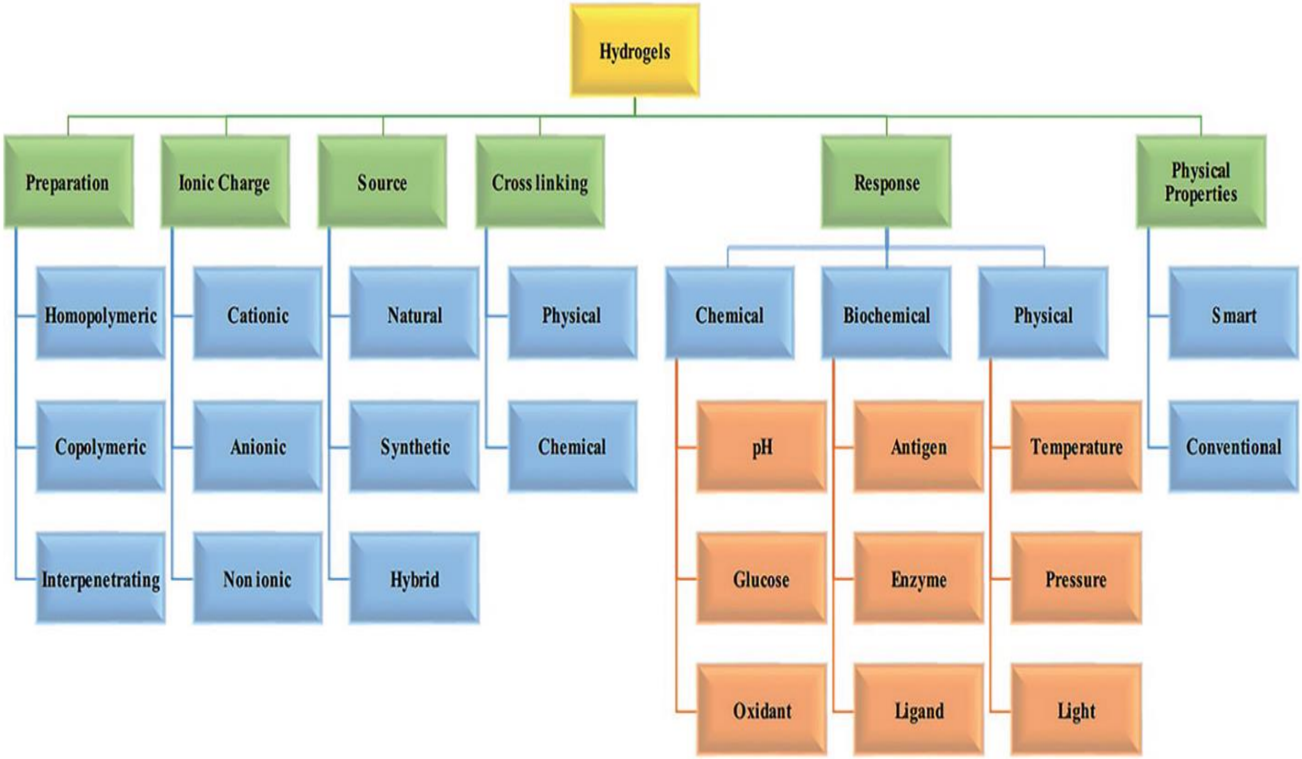

Fig: 1.1 Classification of hydrogels based on the different properties (**Morteza et. al, 2016)**

Several works have been reported in hydrogels, its types and their application in various field. Today hydrogels find a wide range of application because of their lower cost, non toxic, and environment friendly nature.

1.2 Hydrogels in Agricultural field

Polymer that is used in agriculture is gaining traction in research, notably in polymer chemistry. This has brought solutions to current agricultural concerns, such as increasing land and water productivity without endangering the environment or natural resources. Permeability, density, structure, texture, evaporation, and infiltration rates of water through soils could all be affected by superabsorbent polymer hydrogels. Functionalized polymers were utilised to improve the efficacy of pesticides and herbicides, enabling for lower doses to be employed while also protecting the environment indirectly by lowering pollution and cleaning up existing pollutants.**(L.**

O. Ekebafe.et. al, 2011) It has been frequently recommended for agricultural application over the last 40 years with the goal of improving water availability for plants by boosting the water retention characteristics of growing media (soils or soilless substrates)**(T.M. Neethu et. al, 2018).**

1.2.1 Super Absorbent Polymer

In agricultural sector hydrogel polymer can be used as soil conditioners and carriers for slow release fertilizers and protecting agent, hydrogels may be applied by being mixed with the soil or by spraying on the soil surface. (**Waleed Abobatta et. al, 2018)** Agricultural hydrogels are synthetic polymers which absorbs water to improve the soil ability and it is called as Super Absorbent Polymer (SAP) or Super Porous Hydrogels (SPH) **(T.M. Neethu et. al, 2018)**. Prolonged moisture can be obtained by the addition of SPH for a crop to withstand in a certain region (**Ahmad, et. al, 2019)**. SPH incorporating polysaccharides have been widely used, because they appear as viable ecologic and economically alternatives, aiming at soil conditioning on the other hand it a as a constituent-key, serving as a support on polymer network and allowing other properties such as (bio) degradability (**Guiherme et. al, 2015).** When these absorbents mix with the soil, an amorphous gelatinous mass is formed on hydration. They are capable of cyclical absorption and desorption for long period of time. Hence these absorbents act as a slow-release source of water and dissolved nutrients in the soil (**Ramesh Vundavallia et. al, 2020)**

1.2.2 Mode of Action of Polymeric Hydrogels

When the hydrogel is mixed with the soil, it forms an associate amorphous gelatin–like mass on hydration and is adept of absorption and desorption for an extended time, thus acts as a slow unharness supply of water within the soil. The hydrogel particles are also taken as “miniature water reservoir” in the soil and water will be detached from these reservoirs upon the root mandate through osmotic pressure difference **(Chang C et.al,2010)** Due to the respectable volume reduction of the hydrogel as water is released to the plant, hydrogel creates at intervals the soil, free pore volume providing further space for air and water infiltration, storage and root growth. Hence hydrogel polymer deed as a slow–release basis of water and dissolved fertilizers in the soil.

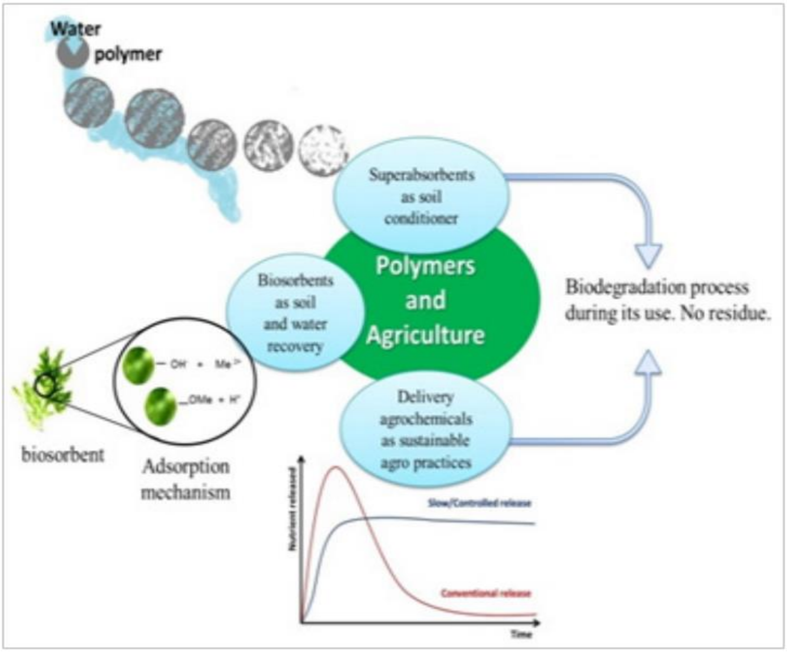

Fig 1.2: Polymer Mechanism in Agriculture (Chang C et. al, 2010)

Water conservation by hydrogel creates a buffered setting being effectiveness in short–run drought tension and losses reduction in institution phase. Ability in water consumption and dry matter production square measure positive crop reactions to hydrogel, once polymers are mixed into soil, they preserved vast quantities of water and nutrients reach up to hundred times of its original weight and conserve regarding ninety–five percent of keep water out there for plant absorption, which are released as required by the plant, therefore, plant growth was enhanced with limited water supply, however, in rainfall region adding hydrogel polymer to soil implement soil infiltration rates. (**K. Sharma, et. al, 2016).**

1.2.3 Effect of Hydrogels in plant growth

Seed germination and seedling establishing considered the most important phases in the initial growth of any plant kinds; the successful establishment depends on available water and is regularly restricted by low level of soil moisture mainly in arid and semi–arid regions. Hydrogel polymers enhancement plant growth by swelling water holding capacity in soil and prolonged the time till reaching wilting point which increasing plants survival under water stress, decreasing fruit drop ratio, and may lead to expanded total yield and fruit weight under various severity conditions. (**H. Khan, et.al,2019)** Furthermore, added hydrogel to the soil increased the plant circumference;

this may be due to increasing the amount of available water in the root zone, which inferring longer irrigation intervals. Moreover, application of hydrogel polymer used to create a water reservoir near the root zone of plants, decrease osmotic moisture of soil, improve the capacity of plant available water, enhancement plant growth and increase whole yield and decrease production costs of crop (Figure 6), uses of hydrogels improving plant viability, seed germination, ventilation and root development mainly under arid environments, additionally, with respect to the growth of the plant, it's been noticed that there's a significant increase in the growth of the plants when usage of the hydrogel**(T.M. Neethu, 2018).**

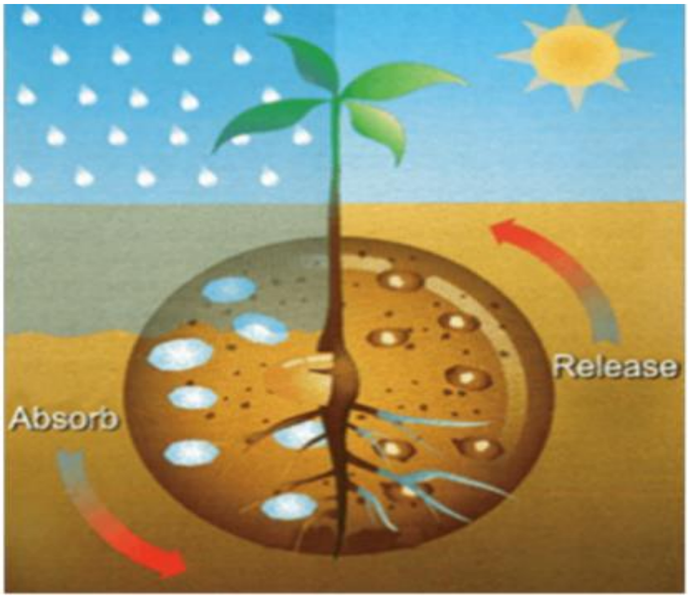

Fig 1.3: Hydrogel swelling water for plants. (H. Khan et. al, 2019)

1.2.4 The Effect of Hydrogel in plant growth

In particular, hydrogel absorbs soluble fertilizer, water and then releases it in proper time for plants.

The effects of hydrogel on soil moister considered as a restricting factor for crop production in arid and semi–arid regions. The polymer as soil conditioners was recognized since the 1950s.

According to (**Hidangmayum A et. al, 2019)** agricultural hydrogels can change the different soil properties through various mechanisms like,

a) Implement water–holding capacity of the soil.

b) Increasing soil permeability.

c) Improving water retention on different soil types.

d) Increase the water use efficiency.

e) Increase irrigation intervals due to increasing the time to reach a permanent wilting point.

f) Minimizing soil erosion and water run–off.

g) Implement soil penetration and infiltration.

h) Decrease soil compaction tendency.

i) Improving soil drainage.

j) Support crop growth performance under reduced irrigation conditions.

k) Enhance nutrient retention as a result of solute release from hydrogel polymer particles and delay the dissolution of fertilizers.

1.3 Swelling behaviour

Hydrogels are made up of networks of crosslinked hydrophilic polymers that inflate rather than dissolve in water (**Peppas et al., 2000**). Because of the charge repulsion across polymer chains, hydrogels consisting of polyelectrolytes swell more, and this swelling property is important in environment-sensitive hydrogels for controlled release in various fields.

SPHs (superporous hydrogels) can swell and shrink rapidly due to capillary forces **(Gemeinhart et al., 2000**). The convection of water into the porous hydrogels causes rapid swelling. SPHs, in particular, have pores.10–1,000 m in diameter, created by gas blowing during hydrogel productiongelation and synthesis (**Kim and Park, 2004).**

Guar gum/poly (acrylic acid) semi-interpenetrating polymer network (IPN) hydrogels has been prepared via free radical polymerization in the presence of a crosslinker of N,N’-methylene bisacrylamide (MBA) by **Xiuyu Li et. al., (2006)**. The kinetics of swelling and the water transport mechanism were studied as a function of the composition of the hydrogels and the pH of the swelling medium. Hydrogels showed enormous swelling in aqueous medium and displayed swelling characteristics, which were highly dependent on the chemical composition of the hydrogels and pH of the medium in which hydrogels were immersed (ionic strength I = 0.15 mol/L). The swelling ratio also depended on the swelling medium pH and in turn affected the liner mesh sizes. The swelling ratios increased with the temperatures of swelling media and the obtained mixing enthalpy indicated the swelling was endothermic process due to the disassociation of the hydrogen bonding.

When dry superabsorbent polymers are immersed in water they swell by two or three orders of magnitude. Both the ultimate degree of absorption and rate of absorption are important in practical applications. Experimentally they depend on the type of polymerisation, e.g. suspension or solution; the monomer composition, e.g. proportions of acrylic acid, sodium acrylate and acrylamide; and on the type of cross-linker, e.g. water or oil-soluble. **H. Omidian et. al., (1998)** investigated the relationships between the various parameters in order to improve understanding and to identify the basic limiting factors. Relationships were also established between the ultimate degree of swelling and the ratio of cross-linker to monomer for the different polymerisation systems. Faster swelling for absorbent made by inverse suspension polymerisation compared with the solution process was attributed to porosity resultant from the formation of the polymer as small particles and intrinsic differences in the nature of the two processes. For the solution and inverse suspension polymerisation, in the presence of MBA as a cross-linking agent, the steady state swelling was inversely proportional to the exponent of about 0.6 of the molar ratio of the crosslinker to monomer.

One of the most important applications of superabsorbent polymers (SAPs), also known as hydrogels, is soil improvement and supporting plant vegetation in agriculture and environmental engineering. Currently, when water scarcity involves water stress, they are becoming still more commonly used for water retention in soil. As it turns out, one of the major factors influencing the superabsorbent polymers water retention capacity (WRC) is the load of soil. **K. Lejcus et. al., (2018)** explained the absorbency under load (AUL) of SAPs. Soil load and bulk densities were simulated by using weights. The experiments were conducted with a Mecmesin Multitest 2.5-xt apparatus. The obtained results demonstrate a very significant reduction in water absorption capacity by SAP under load. One of the solutions may be to use water absorbing geocomposites or other forms of superabsorbent polymers that eliminate or minimise the influence of load on the water absorption process.

SAP conserve water and retain moisture by helping soil increase water holding capacity enabling the survival of plants during drought. They help the environment through soil management, erosion control and environmental cleanups. The amount of available moisture is increased by the use of hydrogels and water stress of plants which is reduced, causing increased plant performance and growth.Hydrogels also claim to reduce leaching of fertilizers. This occurs when the fertilizer interacts with the polymer **(Kris Walker et. al, 2013).**

In terms of super-swelling behaviour SAPs have developed a very appealing sector. As a result, we are increasingly walking in a green environment that is becoming greener by replacing synthetic materials with bio-based components such as polysaccharides and polypeptides. This sets the path for more advancement in this field in the medium and long term

OBJECTIVES OF THE STUDY

- To synthesize Chitosan - Polyvinyl Alcohol Methionine Hydrogel with bisacrylamide as a crosslinker and Ammonium persulphate as an indicator
- To characterize the cross linked hydrophilic polymers in order to correlate the structure and properties of the hydrogel using Fourier Transform Infrared spectra, (FTIR), X-ray diffraction techniques and thermo gravimetric analysis (TG-DTA)
- To study the swelling property and swelling kinetics of the grafted copolymer with various physiological fluids
- To find whether the grafted polymer is stable and efficient in the field of agriculture for further use

2. REVIEW OF LITERATURE

Hydrogels are three dimensional network of hydrophilic cross linked polymer that do not dissolve but can swell in water or can respond to the fluctuation of the environmental stimuli. Hydrogel are highly absorbent natural or synthetic polymeric networks. Hydrogels can be prepared by adding cross linked polymers to water or biological fluids and allowing the mixture to swell. They can be prepared from polymers containing hydrophilic group such as hydroxyl, carboxylic acid, imide, sulphonic acid and amide either embedded in or grafted to their polymeric back bones. Several works have been reported in hydrogels, its types and their application in various field. Today hydrogels find a wide range of application because of their lower cost, non toxic, and environment friendly nature. The aim of this paper is to give an over view of various application of hydrogels in different fields such as Biosensors, Biomedical, Agriculture, Food packaging Industry

2.1 Application of Hydrogels in various fields

Damia Mawad et. al, 2015 reported that due to the specific structures and compatibility hydrogels find a wide range of application in many fields. Flexibility of hydrogels is because of their absorbed water content which makes it possible to use them in different conditions ranging from industrial to biological environments. Hydrogels are majorly used in the fields of food industry, agriculture, biomedical, pharmaceuticals, and biosensors.

2.2 Hydrogel in agriculture

India faces the high risk of water stress and it ranks 41 among 181 countries (**Aniket Kalhapure et. al, 2016).** Nowadays water management is considered one of the major challenges for all countries, there is a fact,that by 2030, global water demand will be probably 50% higher than today, resulting in water scarcity (**Waleed Abobatta et. al, 2018).** In which India uses 70% of fresh water for agriculture so agriculture is under abiotic stress (**T.M. Neethu et. al, 2018)**. Hydrogels are materials with great potential in agricultural applications under the green chemistry approach.The most common hydrogel applications in agriculture are to increase water capacity, to improve the soil structure, as carriers of agrochemicals including fertilizers, pesticides and seed coatings, with the intention to promote crop protection (**Julio Cesar Lopez-Velazquez, et. al, 2019).**

2.2.1 Composities of Agriculuture

L. O. Ekebafe1 et. al, 2011 analyzed that some characteristics of polymers were developed to improve the physical properties of soil

(i) Increasing their water-holding capacity,

(ii) Increasing water use efficiency

(iii) Enhancing soil permeability and infiltration rates

(iv) Reducing irrigation frequency

(v) Reducing compaction tendency

(vi) Stopping erosion and water run-off

(vii) Increasing plant performance

2.2.2 Application of Hydrogels in Agriculture

Hidangmayum et. al, 2019 reported that hydrogel polymers play a vital role in agricultural uses as structural materials for creating a climate beneficial to plant growth in arid and semi–arid regions; it could use as retaining ingredients in different forms as follow:

1) Seed additives to support seed germination or seed coatings.
2) Dipping of seedling roots before establishment.
3) Immobilizing plant growth substances.
4) Coating protecting agents (herbicides and pesticides) for slow release.
5) Polymeric Biocides and Herbicides.
6) Water – insoluble polymers.
7) Polymers for soil remediation.

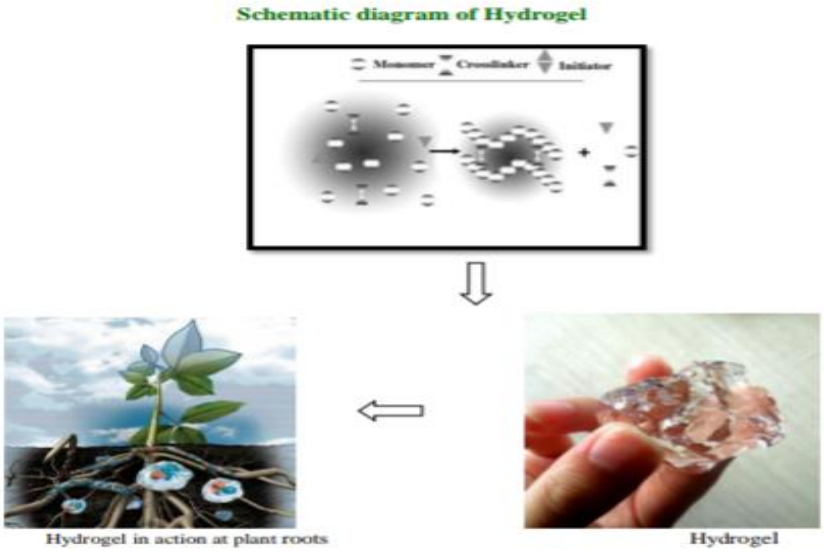

Fig 2.1: Schematic application of hydrogels (**Hidangmayum.A et. al, 2019)**

2.2.3 Agro Chemical Delivery

Agrochemicals such as fertilizers, herbicides, and pesticides which are essential in agriculture. Controlled release techniques that combine agrochemicals with functional polymers will deliver the required agrochemical at its active field into the environment. The desirable properties for agro chemical delivery

(i) Control release of the agrochemicals at the lowest effective concentration,

(ii) Protect the agrochemical from degrading environments such as light and pH,

(iii) Possess lower cytotoxicity than the conventional pesticides, and

(iv)Have long lasting validity of the nano carriers, which will lead to extend the validity period (**Amrita Sikder et. al, 2021**).

C. Demitri et. al, 2013 investigated the potential of cellulose based hydrogels crosslinked with a water soluble carbudiumide to improve agriculture in the aird & desert land. The sustained release of water & efficient storage of the hydrogel was analized. The pilot study of the application of hydrogel was assessed experimentally in green houses for the cultivation of cherry tomato. The study revealed that hydrogels were able to release sustained amount of water to the plant for a prolonged time.

Hisham.A.et. al, 2016 studied Superabsorbent hydrogels made from chitosan-cellulose hybrids by graft polymerization of acrylic acid and their potential for controlled release of soil nutrients. Water adsorption capacity, Fourier transform infrared (FTIR), scanning electron microscope (SEM) and Thermogravimerty analysis (TGA) were used to characterise the hydrogel. The contribution includes increased resilience to acidic environments, increased surface chemical activity, and a wider range of pH responsiveness, all of which contribute to increased water absorbency and retention.These characteristic of the novel superabsorbent made a promising application in controlled release of fertilizers and water in soil

Fatima Sopena et. al, 2007 investigated the alachlor controlled-release formulations to reduce leaching in sandy soils, avoid groundwater contamination, and retain efficacy by using ethylcellulose (EC) under various circumstances to check their mobility. Atrazine-containing agar/starch/poly (AAm) hydrogels were studied by **Baljit Singh et. al, 2015** Scanning electron micrography, Fourier transform infrared spectroscopy, X-Ray diffractometry, and swelling studies were used to characterise these hydrogels. Herbicide was released slowly and according to a non-Fickian diffusion process. These hydrogels have a high water retention capacity and a gradual release of atrazine, making them a promising contender for agricultural applications.

Jamnongkan et. al, 2010 developed controlled-release fertilizer hydrogels based on chitosan and PVA with glutaraldehyde as the crosslinker.The kinetics and mechanic studies in terms of release of potassium fertilizer from the hydrogel was studied. The swelling property was about 70-300% of their original size. The release of potassium was correlated to the swelling ratio of the hydrogel. Higher the swelling ratio higher was the amount of fertilizer released. Due to the boidegradability of chitosan the hydrogel was easily degraded by the soil which releases potassium easily into the soil. Similarly controlled release of urea by using chitosan - salicylaldehyde hydrogel was studied by **Iftime MM et. al, 2019** it was characterized by FTIR spectroscopy, XRD diffraction, POM and SEM microscopy and NMR analysis. It showed a high water absorbency of 68g/g, higher holding capacity of water to the soil by 154 % and nearly doubled the amount of nitrogen content, resulting in 70% higher plant growth.

Table 2.1 Some Application of Hydrogels in Agriculture

S.no	POLYMERIC HYDROGELS	USES IN AGRICULTURE	REFERENCE
1	Anionic herbicide encapsulated in carboxymethyl cellulose (CMC)	Controlled release of herbicides	Sumit Mishra et. al, 2018
2	Chitosan	Crop protection due to its effect on plant response as a alictor	Julio Cesar Lopez-Velazquez et. al, 2019
3	Gelatin	Biostimulant in plant growth	
4	Polyvinyl alcohol (PVA)	Biodegradation of suitably acclimated microorganism	
5	SPH	Prolonged moisture gained in aird areas	Suhail Ahmad et. al, 2019
6.	Chitosan	Induces tolerance to abiotic and biotic stress in various crops	
7.	Polyacrylate	Biefficacy against phytopathogenic fungus	

Recently the use of hydrogel polymer has shown a great potential and growth in the agricultural sector, also, in arid and semiarid regions there is attracting considerable interest for usage of hydrogel polymer to increase soil water retention and improve crop productivity. **(Waleed Abobatta.et. al, 2018)** Hydrogel is a boon to dry farming. Most of the area of India is located in Arid and Semiarid Regions, more efficient use of water is essential in the field of agriculture. Implementing proper management practices in agriculture to maintain soil moisture and increase water holding capacity is considered as one of the ways to save water. Super absorbent polymers (SAPs) hydrogel can swell to absorb huge volumes of water or aqueous solution. This property has led to many practical applications of these new materials in particular in agriculture for improving water retention of soils and the water supply of plants. Especially in the field of slow release nutrients are now being acquired using natural materials. **(T.M. Neethu et. al, 2018)**

2.3 Hydrogels in Biosensors

Parikha Mehrotra et. al, 2016 analyzed the role of hydrogels as Biosensors.

Biosensors are analytical devices that convert a biological response into an electrical signal. Fabrication of biosensors, its materials transducing devices and immobilization methods requires multidisciplinary research in chemistry, biology and engineering

Biosensors started in 1960's by the pioneers Clark and Lyons. Hydrogels are valuable materials for use in biosensors used for immobilization, creating protecting layers controlling diffusion and enhancing biocompatibility.

To achieve this purpose, a biosensor is made up of four main components

i. Bio – receptor
ii. Base material
iii. Transducer
iv. Electronic system

A typical biosensor system includes structural elements, materials, and strategies for sensing. Hydrogel, as a biocompatible polymer with great ability of water absorption, can be used as a base material to form a hydrogel based biosensor.

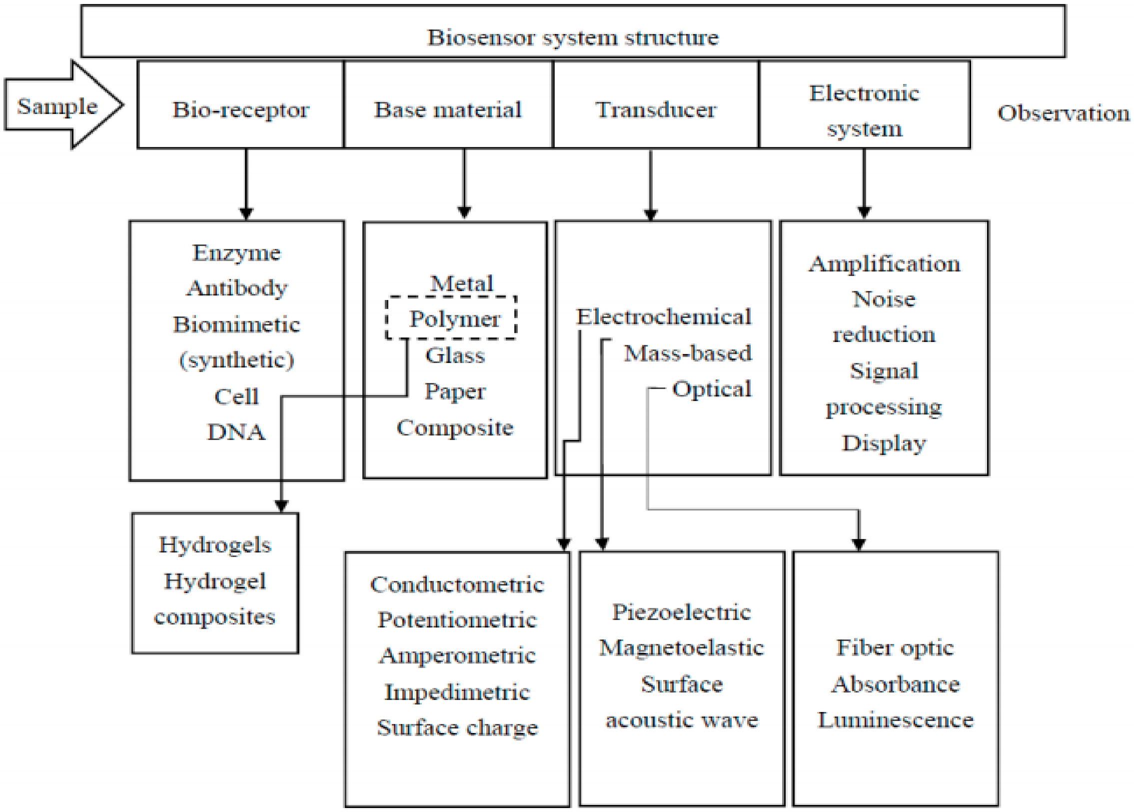

Fig: 2.2: Biosensor system structure (Parikha Mehrotra et. al, 2016)

Polymers are the most popular precursor materials for hydrogel preparation. They are generally employed with matrix to form a stable platform. **Mana Toma et. al, 2013** analyzed that Polyacrylamide (PAAm) is a water-soluble polymer used in water purification facilities. It forms hydrogels easily by absorbing large amount of water molecules. One of PAAm derivaties is Poly (N-isopropylacrylamide) (pNIPAAm) which is used as an alternative material for active control of surface plasmon resonance (SPR) in the visible/near-infrared spectrum. This exhibits a water-swollen structure below LCST and collapses with a release of bound water above LCST.

Javad Tavakoli and Youhong Tang, 2017 have investigated that Polyethylene glycol (PEG) is a hydrophilic biomaterial, with its biocompatibility; PEG is widely used as a biosensor with antifouling characteristics. **Bae J et. al, 2020** have reported that PEG hydrogels have been applied for drug delivery and tissue engineering and that PAAm-based polymers have also been imprinted for monitoring protein crystallization

Susan Mohammadi et. al, 2020 have synthesized carbon dots-chitosan nanocomposite hydrogels for detection of microRNA-21 in MCF-7 cancer cells by reacting carbon dots generated from different aldehyde precursors with chitosan and then functionalizing with ssDNA probe. UV-vis absorption and fluorescence spectra, FT-IR, scanning electron microscope (SEM) and transmission electron microscopy (TEM) were the techniques used for characterization of the hydrogel. This Hydrogels are suitable for sensing and biosensing platforms because they have multicolor fluorescence, great mechanical strength, high adhesiveness, and strong antibacterial characteristics.

Table 2.2 Some Applications of Polymeric based Hydrogels in Biosensors

S.no	HYDROGELS	USES	REFERENCE
1.	PAAc-co-polyacrylamido-methyl propane sulfonic acid copolymer hydrogel	Determines the presence of transition metal ions	Joonwon Bae, et. al, 2020
2.	dimethylaminopropylacrylamide hydrogel using 3 phenylboronic acid and a tertiary amine	monitoring of glucose in blood	
3.	poly-2-hydroxyethyl methacrylate (PHEMA) hydrogels	Amperometric determination of nitrite	
4.	Polyacrylamide incorporated with glass carbon electrode	Determination of toxic substances	
5.	Polyvinyl alcohol	Detection of Escherichia coli	
6.	Polyethylene Glycol (PEG)	Drug delivery and tissue engineering	
7.	poly-N-isopropylacrylamine (PNI PAAm	Immobilizing DNA	
		Detection of trace amounts of molecular analytes	Mana Toma et. al, 2013
		Sensing of pH and temperature in a biosystem	Javad Tavakoli and Youhong Tang, 2017
8.	Polyethylene Glycol (PEG)	Biosensor with antifouling characteristics	
9.	Polyvinyl alcohol	Wound dressing or tissue engineering	Fuli Zhao et. al, 2015

Conducting polymers are also called as synthetic polymers. It is a carbon-based polymer which are electronically active and being investigated for fabrication of electroactive hydrogels. **(Damia Mawad et. al, 2015).** Conducting polymers showed excellent properties such as electrical properties, biocompatibility, mechanical flexibility, and ease of processing. **(Bin Guo et. al, 2019)**. Conductive materials were introduced by two methods they are polymerization inside hydrogel as precursors and mixing. (**Joonwon Bae et. al, 2020).**

Conductive hydrogels polyvinyl alcohol/glycerol/polyaniline (PGA) was synthesized by **(Chengxin Hu et. al, 2018)**. PGA conductive gel showed good electrical conductivity and mechanical characteristics. The gel can also be recovered using a three-step technique that includes heating injection, chilling, and cyclic freeze-thaw cycles. At subzero temperatures, the PGA conductive gel is expected to be exploited as an unique multifunctional material in a variety of applications. A biosensor developed with PGA conductive gel was utilized to evaluate the normal motion signal of the human body with high sensitivity, quick response time, and numerous use cycles.

The PANI grafted PAM-PVA polymer composite loaded urease modified electrode was proposed by **Joyati Das et. al, 2016** as a urea sensor. Scanning electron microscopy (SEM), atomic force microscopy (AFM), Fourier transform infrared spectroscopy (FTIR), Electrochemical impedance spectroscopy (EIS), differential pulse voltammetry (DPV) and cyclic voltammetry (CV) were the techniques used for characterization of the hydrogel. The hydrogel showed good detection limit, sensitivity, Km value. The preparation technique, cost, stability and selectivity of the polymer made it possible to propose it as high performance sensor.

Table 2.3 Some Application of Conductive based Hydrogels in Biosensors

S.no	HYDROGELS	USES	REFERENCE
1.	Polyaniline	Senses glucose	Lanlan Li et. al, 2012
2	Poly(ethylenedioxythiophene)	Drug delivery , soft strain sensors	Damia Mawad et. al, 2015

3.	Polypyrrole	Clinical diagnosis	Fuli Zhao et. al, 2015
		Determination of ascorbic acid, dopamine, and uric acid	Joonwon Bae et. al, 2020
4.	Graphene oxide based hydrogel	Acts as a support for biological oxygen demand (BOD)	
5.	Polyaniline	Dection of xanthine	

Carbohydrates are considered most desirable for hydrogel formation, because they are naturally abundant and environmentally benign. Carbohydrates such as oligosaccharides, polysaccharides, cellulose, chitosan, and dextran are suitable for hydrogel generation because the molecular units, are composed of hydrophilic moieties. Hence, they can sufficiently absorb water molecules inside the molecular network. Sometimes, they can transform their configuration to adjust to the environment. Therefore, carbohydrates are widely used for hydrogel preparation. Compared with other hydrogel precursors, carbohydrates are less miscible with other components and show poor chemical/mechanical stability. (**Joonwon Bae et. al, 2020)**

For the detection of thrombin, a "signal-on" chemiluminescence biosensor was developed by **(Yanna Lin et. al, 2019)** the biosensor successfully isolates and enriches thrombin with a biocompatible MalgApt1 and was easily recognised**.**

The use of DNA and peptides as auxiliary components in hydrogel production is common. According to **(Eleonora Parelius Jonasov et. al, 2016)** unique interactions between DNA/peptides and ions/small molecules can be used as a sensitive detection technique. The signal creation was dependent on precise interactions between DNA/peptide and target analytes, the sensing mechanism's fidelity was low.As a result, substantial study is occurring in a variety of sectors. A heavy metal ion, for example, was also detected using DNAfunctionalized hydrogels.

For the detection of Pb^{2+}, a portable DNA-based hydrogel capillary sensor (DHCS) was effectively constructed by **(Chao Jiang et. al, 2019).** The hydrogel film controls the capillary action of the capillary tube, and the mesh size of the hydrogel film is determined by the Pb^{2+} concentration. As a result, this technique had the advantages of being simple, cost-effective, ubiquitous, easy to read out, and very sensitive for detecting Pb^{2+}, indicating that it had promising applications in food safety, environmental monitoring, molecular diagnostics, and other fields.

The development of a DNA hydrogel-based electrochemical impedance biosensor for Hg^{2+} detection has been discovered by **Wei Cai et. al, 2017**. The biosensor is capable of reliably and quantitatively discriminating Hg^{2+} ions, indicating that this approach has promise for preliminary use in regular assays. As a result, the synthesized biosensor has a high potential for reliably and quantitatively discriminating Hg^{2+} ions in complex river water samples.

Table 2.4 Some Application of Biological origin based Hydrogels in Biosensors

S.no	HYDROGELS	USES	REFERENCE
1.	Carbohydrate	Prevent migration of the microgels from target sites	Javad Tavakoli and Youhong Tang et. al, 2017
2.	Cellulose	shows a pH and salt sensitive swelling behavior	
3.	Peptide cross-linked hydrogel	Tissue engineering; response to diverse biological stimuli	
		Direct monitoring indicator for matrix metalloproteinase	Bae J et. al, 2020
4.	Oligosaccharide hydrogel	Monitoring alpha-amylase	
5	Chitosan hydrogels on a glassy carbon electrode	Measurement of lactate in beverages and dairy products	
6	DNA functionalized hydrogels	Heavy metal ions can be detected	

Recently, research activities regarding hydrogel based biosensors have been actively pursued in many Journal Pre-proof relevant fields. There are many stimuli in the ambient environment, such as light, heat, pressure, pH, and pathogens. Stimuli-responsive materials are attractive because they can modify the structure, property, or geometric configuration depending on a change in physical parameters such as temperature and pressure, and chemical conditions such as pH and ionic character, electrical properties, and environment. As it is convenient to obtain biosensors by observing any visual changes in color, research regarding colorimetric and fluorescence sensors is active. **(Jones AM et. al, 2013)** The identity of each hydrogel disk sensor was determined using pattern-recognition software. A 98% recognition accuracy was demonstrated with non defective sensors. This new method for generating ar-rays of hydrogels for biosensing would be beneficial for quick and inexpensive biochemical testing.(**Eleonora Parelius Jonasova et. al, 2016)**

Until now, the incorporation of CPs in a prefabricated hydrogel network has dominated the field of conductive hydrogels. Advances in fabrication techniques have enabled smart multifunctional conductive hydrogels with tailored architectures to promote cell alignment, release of biomolecules under demand, and growth and differentiation in response to electrical stimulation Additionally, chemical binding or self-assembly of the CPs chains results in a more stable network that is resilient to environmental changes such as pH. **(Bae J et. al, 2020)**

2.4 Hydrogels in Food Industry

Food is indispensible for the sustaining life process in all living creatures. In recent years, rapid industrialization, population growth, and changed life style lead to increased demand for processed and packed foods **(Mahendra Pal et. al, 2019)**.Plastic packaging is essential nowadays. However, the huge environmental problem caused by landfill disposal of non-biodegradable polymers in the end of life has to be minimized and preferentially eliminated. Food packaging is essential for products containment, protection, preservation, convenience, to provide information about the product, brand communication, among others. **(Ana R. V. Ferreira et. al, 2016)**. Bio based polymeric hydrogels have been introduced as an alternative approach to deal with the problem of disposal of waste plastic packaging material **(Niladri Roy et. al, 2012).** However, the use of superabsorbent polymers as bio-based hydrogels in the food industry remains only limited. (**Batista RA, et. al, 2018).**

2.4.1 Biodegradable Polymers

Biodegradation is a chemical degradation of materials provoked by the action of microorganisms such as bacteria, fungi and/or algae. According to the definition, a biodegradable polymer is "a degradable polymer wherein the primary degradation mechanism is through the action of metabolismby microorganisms".(**Niladri Roy et. al, 2012)**

Ana R. V. et. al, 2016 classified biopolymer according to their source

1. Polymers directly extracted/removed from biomass such as polysaccharides (e.g.,starch,cellulose, and galactomannans) and proteins (e.g., casein and gluten).
2. Polymers produced by chemical synthesis from renewable bio-derived monomers, such as polylactic acid (PLA), a thermo plastic aliphatic polyester derived from lactic acid monomers. The monomer itself is produced via fermentation of carbohydrate feedstocks.
3. Polymers produced by microorganisms ,like some polysaccharides (e.g.,gellan gum and pullulan) and polyhydroxyalkanoates (PHA)

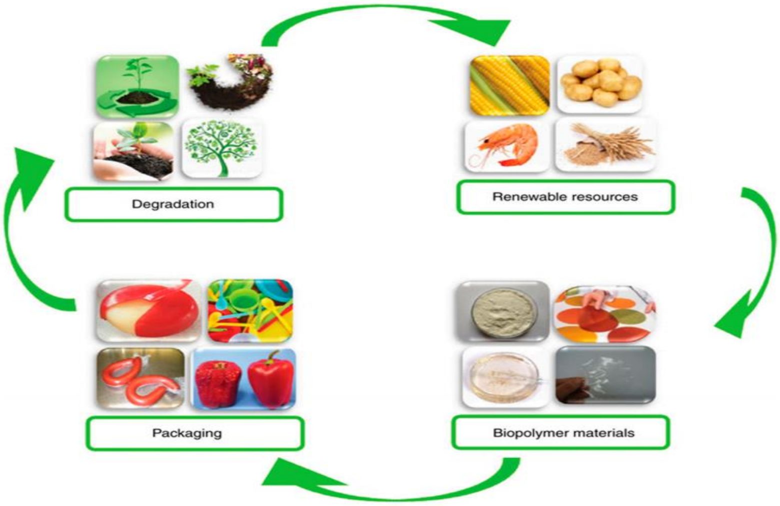

Fig 2.3: Life Cycle of Biopolymer Packaging Materials.(Niladri Roy et. al, 2012)

2.4.2 Polysaccharides in Food Packging

Polysaccharides are the most abundant macromolecules in the biosphere. A high variety of polysaccharides and their derivatives have been used to produce biodegradable films and thin membranes. Polysaccharide-based membranes have been widely used in food industry in packaging and edible coatings

Table 2.5 Main Polysaccharides for Constructing Hydrogels and their origin

S.No	SOURCE	POLYSACCHARIDE	REFERENCE
1	Plant	Starch, pectin, cellulos, Gum Arabic (gum acacia), gum karaya, gum ghatti, gum tragacanth, Guar gum, locust bean gum (LBG), tara gum, tamarind , xyloglucan Konjac glucomannan	Hongbin Zhang et. al, 2020
2	Animal	Chitosan, Chitin	
3	Microbial	Xanthan gum, gellan gum, curdlan, dextran, bacterial cellulose	
4	Algal	Agar (agarose), carrageenan, Alginate	

2.4.3 Application of Polysaccharide obtained from plant, animal and algae in Food Industry

(Niladri Roy et. al, 2012) studied about polyvinylpyrrolidone (PVP) and biopolymer (carboxymethyl cellulose (CMC) and its biodegradability under specified environmental conditions to create a hydro-gel film as an alternative to the use of plastic as a food packaging material**.** The biodegradation of PVP–CMC hydrogel films was examined until 8 weeks in a liquid state.Within 8 weeks, a 38 percent weight loss was recorded. Fourier transform infrared (FTIR), scanning electron microscope (SEM) were used to characterise the hydrogel. PVP:CMC= 20:80 hydrogel film is mechanically strong and flexible, according to the results. This hydrogel coating could absorb the released moisture and keep the environment dry, preventing food materials from decaying quickly and maintaining oxygen diffusion.

Hydrogel-forming carbohydrates including agar, alginate, and collagen, as well as fillers like AgNPs and GSE, were used to create ternary blend biohydrogel films and composite films by **(Long-Feng Wang et. al, 2015)**. The water capacity of the prepared films was 23.6 times their weight, the produced films demonstrated excellent antibacterial efficacy against foodborne pathogens Listeria monocytogenes (gram-positive) and Escherichia coli are harmful microorganisms (gram-negative). Furthermore, the GSE and AgNPs included films can be used as an active food packaging material to prolong the shelf life of food goods while also maintaining quality by reducing post-processing contamination.

Table 2.6 Application of Polysaccharide obtained from plant, animal and algae in Food Industry

S. No	POLYSAC CHARIDE	COMPLEX	USES	REFERENCE
1	Chitin	N-acetyl glucosamine	Coffee capsules, Food bags Packaging films	Ana R. V. Ferreira et. al, 2016
2	Chitosan	D-glucosamine N-acetyl-Dglucosamine	Edible membranes and coatings (Strawberries, cherries, mango, guava, among others) Packaging membranes for vegetables and fruit	
3.	Galactomannans	Mannose Galactose	Edible membranes and coatings in Fruits ‘ Cheese	
4.	Cellulose	Glucose	Cellophane membranes	
5.	Carrageenan	Galactose	Coatings Fruits Meet Encapsulation of aroma compounds	
6.	Alginate	Mannuronic Glucuronic acid	Coatings Prevent water loss in fresh cut fruit (apple, papaya, pear and melon) Inhibition of microbial growth (turkey products) Microwaveable food (increase warming efficiency)	
7.	Starch	Glucose	Flexible packaging: Extruded bags, Nets for fresh fruit and vegetables, Rigid packaging, Thermoformed trays and containers for packaging fresh food	
8.	Alignate	Gelatin	Food packaging	Long Feng Wang and Jong Whan Khim, 2015

9.	Starch	Citric acid / gelatin	Moisture absorption	Patronela Nechita et. al, 2020
10.	Cellulose	Ester (cellulose acetate)	Edible food packaging	Aleksandra Nesic et. al, 2019
11.	Carrageenan	Polylactide (PLA)	Improved Barrier	
12.	Chitosan	Poly (butelene succinate)	packaging membrane	Patricia Cazon et. al, 2017
13.	Cellulose	Methyl cellulose	Oxygen, carbondioxide and lipid barrier	
13.	Starch	Poly vinyl alcohol	Maintains biodegradability	

2.4.4 Polysaccharides obtained from Microorganism

Several polysaccharides with film-forming ability can be produced by microorganism (yeast,fungusorbacteria), suchaspullulan, gellangum, xanthangum, FucoPol, bacterial celluloseorbacterial alginates

Table 2.7 Application of Polysaccharide from Microorganism used in Food Industry

S. No	POLY - SACCHARIDE / MICRO ORGANISM / COMPLEX	USES	REFERENCE
1	Pullulan / Aureobasidium pullulans / Maltotriose (three glucose)	Coating material, Wrapping material Blends with other polymers to improve mechanical properties, Inner package , Seasoning bag of instant noodles , Instant coffee	Aleksandra Nesic et. al, 2019
2	Gellan gum / Sphingomonas elodea / Glucose Rhamnose Glucuronicacid	Edible Coatings in breading and batters for chicken, fish, cheese, vegetables and potatoes. Encapsulation of flavor and bioactive ingredients	Hongbin et. al, 2020
3	Xanthan gum / Xanthomonas campestris / Glucose Mannose	Glucuronicacid AcetatePyruvate Edible coating , Meet (Prevent moisture migration during frying) , Fruit (Extend shelf-life)	Long Feng Wang and Jong Whan Khim, 2015
4	FucoPol / Enterobacter A47 / Fucose Galactose Glucose Glucuronicacid Acetate Succinate Pyruvate	Possible application as inner layer in multilayer packaging	Heather M et. al, 2013

2.4.5 Protein based Polysaccharides

Proteins are macromolecules that naturally display different sequences of amino acids that are combined by peptide bonds. For protein film formation, chain alignment and structure highly depend on the present arrangement of amino acids **(Markus Schmid and Kerstin Muller, 2019).** Covering foods with edible films can effectively prevent the loss of moisture and flavors, control gas (oxygen, carbonic dioxide and ethylene) exchange and transport active substances (e.g. antimicrobials, antioxidants, or nutraceuticals).The excellent gas barrier qualities and satisfactory mechanical properties make the protein-based biopolymers one of the most potential materials for food packaging.

Leila Yavari Maroufia et. al, 2021 discovered to use the Schiff base reaction to evaluate the synthesis and structural features of a chitosan (CS) – modified dialdehyde guar gum (DAGG) hydrogel. The greatest swelling capacity was obtained around 12,000 percent at a 30:70 mixing ratio of the hydrogel. Gel strength of a hydrogel is a critical aspect in its performance in food packaging. As a result PPE-loaded hydrogels has been proven to be a potentially acceptable material for absorbing moisture at the bottom of trays of fresh beef, chicken, and fish packets in order to extend their life span.

Edible active films.were synthesized by **Danyxa Piñeros-Hernandez et. al, 2016** using cassava starch, glycerol, and natural polyphenols derived from rosemary leaves. The addition of rosemary extract to glycerol plasticized cassava starch films improved the films' UV-blocking abilities. The findings imply that rosemary extract, as a natural antioxidant, has a high potential for producing active and biodegradable cassava starch films.

Table 2.8 Application of Protein based Polysaccharide in Food Industry

S.NO	HYDROGELS	USES	REFERENCES
1	Beta-lactoglobulin/pectin	Food wrapping	Christophe Schmitt et. al, 2011
2	Starch/cellulose	Food packaging	Niladri Roy et. al, 2012
3	Chitosan/gelatin	Antimicrobial packaging	Gregorova, A et. al, 2014
4	Gelatin/pectin	Food covering	Ana R. V. Ferreira et. al, 2016
5	Methylcellulose/whey protein	Moisture sensitive packaging	Mahendra Pal et. al, 2019
6	Zein/starch	Inner packaging	Santosh Kumara et. al, 2020

Intensive academic and industry research is being carried out to find new and improved polymers, production methods, sources and properties, to obtain biopolymers (in particular, polysaccharides) that may replace the conventional synthetic and non-biodegradable ones as packaging materials. (**Santosh Kumara, Avik Mukherjeeb, Joydeep Dutt.et. al, 2020)** The future trends are related with industrial development, able to produce competitive products in performance and price

2.5 Hydrogels in Biomedical and Drug Delivery

Hydrogels have been used as a carrier in delivery of drug for several decades to reduce the drawbacks associated with traditional drug delivery. Because of their biocompatibility, physicochemical qualities, and designed interaction with living environments, they have been regarded as one of the most dependable classes of biomaterials. Hydrogels' adaptability and diversity make them unique not just in the field of targeted drug delivery, but also in tissue engineering, contact lens production, and wound dressings. Hydrogels made from natural, semi-synthetic, and synthetic polymers have received a lot of attention in recent years for targeted medication delivery of therapeutic agents for cancer, gastric ulcers, brain tumours, diabetes, bacterial infections, and other diseases**.**

2.5.1 Multiscale properties of hydrogels

Hydrogels vary in size, design, and function and these characteristics influence how they are employed for drug delivery. The characteristics in hydrogels vary with length scales ranging from centimetres to microns (Fig 2.4). The routes by which hydrogels can be transported into the human body are largely determined by the macroscopic design. **(A. Pourjavadi .et. al, 2015**)

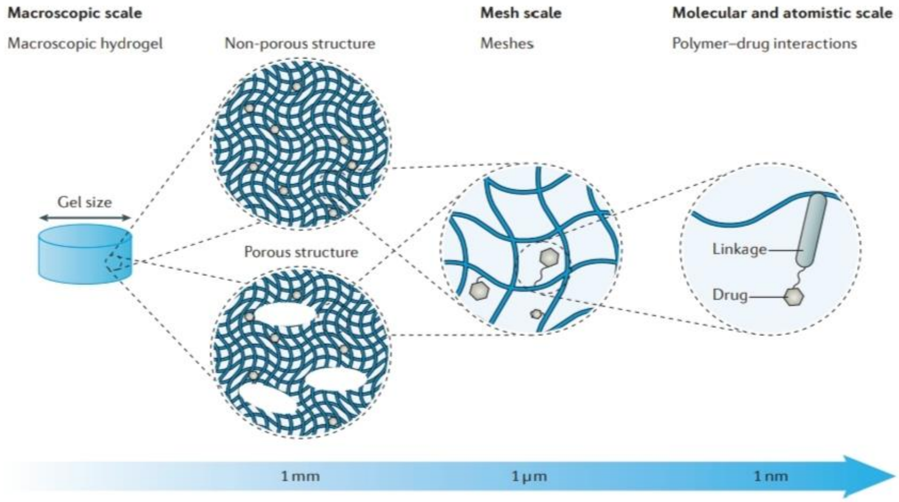

Fig 2.4: The macroscopic design of hydrogels includes the size and porous structure.

Hydrogels can be either non-porous or contain macroscopic pores that are 10–500μm in size. The spacing between polymer molecules in the network (that is, the mesh size) is tunable from around 5 to around 100nm. At the molecular (or atomistic) scale, drugs can interact with the polymer chains via a range of mechanisms; shown here is a covalent linkage to a polymer chain

2.5.2 Design and Deliver Route

The size of a hydrogel is significant. Hydrogels can be cast or moulded into almost any shape or size depending on the delivery route into the human body. There are three types of hydrogel delivery systems.There are three primary groups based on size: macroscopic microgels, nanogels, and hydrogels (Fig.2.5). Microgels and microparticles Nanogels are particulate hydrogels with diameters on the order of nanometers**.(** J. Li **.et.al,2016)**

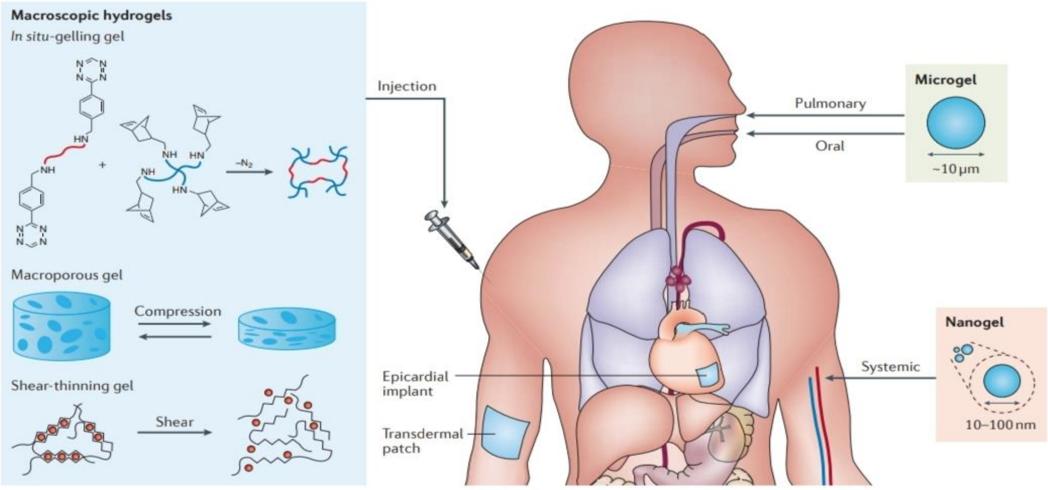

Fig 2.5: Macroscopic design determines the delivery route.

Macroscopic hydrogels are used for transepithelial delivery and placement inside the body. Injectable macroscopic hydrogels that can be delivered via syringe-needle injection include in situ-gelling hydrogels such as a hydrogel formed with tetrazine-norbornene chemistry, shear-thinning hydrogels such as alginate hydrogels crosslinked with multivalent ions and macroporous hydrogels that can undergo reversible dramatic volumetric change. In addition to transepithelial and local injection, microgels are suitable for oral, pulmonary and intrabony delivery and nanogels are suitable for systemic administration of drugs.

2.5.3 Application of Hydrogels in Biomedical and Drug Delivery

(K. Park et al, 1993) described the biodegradable hydrogels advantages in the area of drug delivery systems. Covalently cross-linked synthetic hydrogels, covalently cross-linked hydrogels made of natural polymers, covalently cross-linked hydrogels with degradable crosslinking agent, non-cross-linked biodegradable hydrogel system, Non-mediated hydrogels which have the biodegradable properties have been studied. He concluded that the release of entrapped drugs can be controlled by e altering the degradation rate of the hydrogel.

Y. Qiu, K. Park. et al, 2001 investigated hydrogels can protect drugs from potentially harmful environments, such as enzymes and low pH in the stomach. By changing the gel structure in response to environmental stimuli, hydrogels can also control drug release. Hydrogels with such 'sensor' properties can go through reversible volume phase transitions or gel–sol phase transitions in response to even minor changes in the environment. Environmentally sensitive hydrogels are also known as "intelligent" or "smart" hydrogels. The smart hydrogel systems have been exposed to a variety of physical and chemical stimuli in order to elicit various responses. Temperature, electric fields, solvent composition, light, pressure, sound, and magnetic fields are examples of physical stimuli, while pH, ions, and specific molecular recognition events are examples of chemical or biochemical stimuli.Smart hydrogels have been used in a variety of applications, including the creation of artificial muscles, chemical valves, enzyme and cell immobilisation, and bio separation. Hydrogels that are sensitive to the environment are excellent candidates for developing self-regulating drug delivery systems.

M. Bansal, et al, **2020** have described the fabrication of interpenetrating CPHs supported by a hydrogel network, while others describe pure CPHs in which CP monomers are modified to form a hydrogel network on their own. A stable adhesion of the CPH layer on the under-lying electrode, as well as covalent anchoring strategies, is required for the performance of an electrically responsive drug delivery device. CPHs have emerged as a promising drug delivery option, offering several advantages over other electro-conductive hydrogels.

Hydrogels are widely applied in the field of medicine such as sensing and actuating. Because of its soft nature it has excellent applications in efficiency of other structures. It is evident that the complexity of the devices and applications is increasing while research in this field is mature enough to start experimenting with advanced structures. As a result, it can be safely predicted that hydrogel devices will continue to be highly involved in biomedical research and applications in the future**. (Kosmas Deligkaris et. al. 2019)**

Applications and recent research on hydrogels in various fields were briefly discussed. Although hydrogels have existed for more than half century, still hydrogels fascinate material scientists and biomedical researchers and great strides have been made in terms of their formulations and applications. Despite all their beneficial properties, there are still several challenges to overcome for clinical translation. There are numerous patents on hydrogels but only a few reached the market. Thus, hydrogels can be appreciated for opening up new frontiers in various application in research field

3. MATERIALS AND METHODS

3.1 General:

The materials, instruments and apparatus used, preparation of various reagents and chemicals by various methods are discussed below.

3.1.1. Chemicals and reagents used:

1. Polyvinyl Alcohol (PVA)
2. L-Methionine
3. Chitosan – from shrimp shells 75% (deacetylated)
4. N, N'- Methylene bisacrylamide
5. Ammonium Persulfate
6. 12.5 % Glacial Acetic acid
7. 1 N NaOH

3.1.2 Synthesis of Chitosan Poly Vinyl Alcohol Methionine Hydrogel [CSPVAM]

PVA is grafted with L-methionine (10:1) using ammonium persulphate in oxalic acid medium at 0-5 ^{o}C. The formed grafted PVA (PVAM) is precipitated out using acetone. PVAM is dried and used for crosslinking with chitosan.

5g of chitosan is weighed and added to a 500ml beaker. 100ml of 12.5% glacial acetic acid [12.5 ml of acetic acid + 87.5 ml of water] is added into the beaker and placed in magnetic stirrer for 4hr. After constant stirring 2g of PVAM, 0.1g of N, N'- Methylene bisacrylamide, 0.5g of Ammonium Persulfate is added to the mixture with constant stirring and allowed to stir for overnight. Chitosan Poly Vinyl Alcohol Methionine Hydrogel [CSPVAM] is obtained.

Fig 3.1: CSPVAM Hydrogel solution

3.1.3 Preparation of CSPVAM hydrogel beads

A small amount of 1 N NaOH [10 g of NaOH in 250 ml of water] is taken in petridish. With the help of dropper small drops of CSPVAM hydrogel is placed in the NaOH solution and allowed to stand for 2 hrs. After 2 hrs CSPVAM hydrogel beads are taken out from NaOH solution, washed in distilled water. The beads are dried in preheated hot air oven to about 60^{o}C[and turned off] for overnight. The dried flakes of CSPVAM hydrogel are obtained.

Fig 3.2: CSPVAM hydrogel beads

Fig 3.3: CSPVAM hydrogel flakes

3.2 Characterization

3.2.1 Fourier Transform Infrared Spectroscopy

The synthesized graft polymer CSPVAM hydrogel have been characterized by Fourier transform infrared spectral techniques. To find whether the polymers have been grafted in a proper method. Infrared spectra of graft polymers were recorded using Perkin Elmer FT-IR spectrometer with the SOFTWAREE – OPUS version 6.5.

3.2.2 Powder X-ray diffraction

X-ray diffraction spectroscopy as a rapid analysis technique is used to identify the type of material as well as its phase and crystalline properties in hydrogels .High quality powder X-ray diffraction (PXRD) data were collected in diffractometer system XPERT – PRO over an angle range of 5 to 60^{o} in 2θ.

3.2.3Thermogravimetry and Differential Thermal Analysis

The thermal degradation and stability of the CSPVAM hydrogel were studied using TG/DTA - EXSTAR/6300 simultaneous thermal analyzer. It is used to analyze the endothermic behavior in the phase transition when the volume phase transition of the hydrogels occurs; meanwhile, the weight loss resulting from water loss in the whole process can also be observed. Alumina was used as a reference material for thermogravimetry and differential thermal analysis of the hydrogels.

3.3 Swelling Kinetics

3.3.1 Preparation of salt solution for kinetic studies

To study the swelling behavior of hydrogel 5 sets of salt solution with 3 different level of concentration is prepared

Table 3.1: Different concentrations of salt solution

Salt solution (100 ml)	**0.1 M**	**0.3 M**	**0.5 M**
NaCl	0.5844 g in 100 ml of water	1.7532 g in100 ml of water	2.922 g in 100 ml of water
KCl	0.7460 g in 100 ml of water	2.2360 g in 100 ml of water	3.730 g in 100 ml of water
KBr	1.1900 g in 100 ml of water	3.5700 g in 100 ml of water	5.950 g in 100 ml of water
KI	1.6600 g in 100 ml of water	4.9800 g in 100 ml of water	8.300 g in 100 ml of water
$CaCl_2$	1.1098 g in 100 ml of water	3.329 g in 100 ml of water 4	5.549 g in 100 ml of water

Based on the 5 sets of salt solution the CSPVAM hydrogel flakes are weighed and taken in the tea bag and dipped into the solution of 3 different concentrations for 3hrs. Then the tea bag was taken out from the solution and wiped to remove the surface water and reweighed to calculate the water absorbance.

Fig 3.4: Tea bags (from Semeha Exports) used for Swelling Studies

3.3.2 Preparation of physiological fluids for kinetic studies

4 sets of physiological fluids were prepared and studied the swelling behavior of hydrogels in different intervals of time

1. Urea : 5g in 100 ml of water
2. Glucose : 5g in 100 ml of water
3. Saline water : 0.9g of NaCl in 100 ml of water
4. Synthetic urine : 0.8g of NaCl + 0.1g of $MgSO_4$+ 2g of Urea + 0.06g of $CaCl_2$

Table 3.2: Time intervals for physiological fluids

Physiological fluids	**Time intervals**		
Urea	12hrs	24hrs	6 hrs studies at regular interval of 1hrs
Glucose	12hrs	24hrs	6 hrs studies at regular interval of 1hrs
Saline water	12hrs	24hrs	6 hrs studies at regular interval of 1hrs
Synthetic urine	12hrs	24hrs	6 hrs studies at regular interval of 1hrs

Based on the 4 sets of Physiological fluids the CSPVAM hydrogel flakes are weighed and taken in the tea bag and dipped into the solution for different intervals of time. Then the tea bag was taken out from the solution and wiped to remove the surface water and reweighed to calculate the water absorbance.

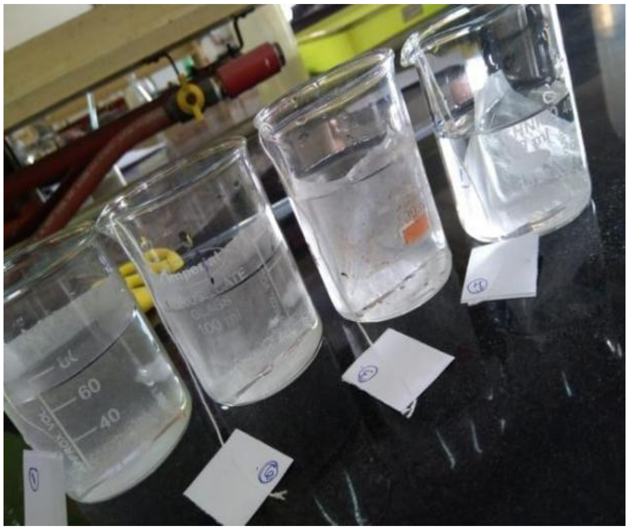

Fig 3.5: CSPVAM hydrogel beads immersed in Physiological fluids - Swelling Studies

3.3.3 Swelling studies at different pH levels

Swelling behavior of CSPVAM hydrogel were studied by maintaining pH at different levels by using 1 N NaOH and 1 N HCl.

Table 3.3: Hydrogels at different pH levels

Hydrogel	**pH levels**
CSPVAM hydrogel	pH 3
	pH 4
	pH 7
	pH 8
	pH 10

CSPVAM hydrogel flakes are weighed and taken in the tea bag and dipped into the solution with different pH levels for 1 hrs. Then the tea bag was taken out from the solution and wiped to remove the surface water and reweighed to calculate the water absorbance.

3.3.4 Measurement of water absorbency- Swelling Kinetics

The following formula was used to calculate the water absorbency capacity:

$$Swelling - percentage = \frac{W_2 - W_1}{W_1} * 100 \tag{1}$$

$$Swelling - ratio = \frac{W_2 - W_1}{W_1} \tag{2}$$

Where W_1 and W_2 are the dry and swollen sample weights, respectively. The swelling kinetics was investigated by repeating the same tests at different times. The water absorbency of the grafted copolymer was calculated using the increase in weight of the swelled sample.

4. RESULTS AND DISCUSSIONS

The CSPVAM was characterized and its swelling responses were studied in for various physiological liquids. The results pertaining to these experiments are discussed under the following headings.

- ➢ Synthesis of CSPVAM hydrogel
- ➢ Characterization of CSPVAM hydrogel
    - FTIR Spectroscopy
    - XRD Studies
- ➢ Stability of CSPVAM hydrogel
    - TG/DTA Thermal Analysis
- ➢ Swelling Responses of CSPVAM hydrogel
    - In Different Salt Solution
    - In Physiological Solutions
    - In Distilled water for various immersion time
    - In Distilled water for 3 hrs in different pH

4.1. Synthesis of PVAMBis Hydrogel

The amino acid L-methionine was grafted on to the PVA backbone by the solution polymerization of PVA and methionine using ammonium persulfate as an initiator. The formed PVAM graft polymer was cross linked with chitosan by the use of cross linker N,N’-methylene bisacrylamide, to get CSPVAM hydrogels. The so formed CSPVAM hydrogel beads are formed by adding drops on to 1 M NaOH solution. The hydrogel beads are dried and used for characterization and swelling studies.

4.2. Fourier Transform Infrared Spectra

The IR spectra of pure PVA, Chitosan and CSPVAM is shown in Figure 6. The FTIR spectrum of pure PVA showed the characteristic absorption bands for O-H, C-H and C=O/CO peaks. The band observed between 3500 and 3200 cm^{-1} are ascribed to the O-H stretch vibration. This band appeared as a broad one due to intermolecular and intramolecular hydrogen bonding in

PVA chains. PVA showed vibration band at 3000-2800 cm^{-1} and was associated with C-H stretching vibration of the alkyl group. The absorption corresponding to C=O was present in the region of 1750-1960 cm^{-1}. In addition to these bands, it also has peaks due to C-H bending and deformations in the finger print region 900-500 cm^{-1}(**Wu, J. et. al. 2012).**

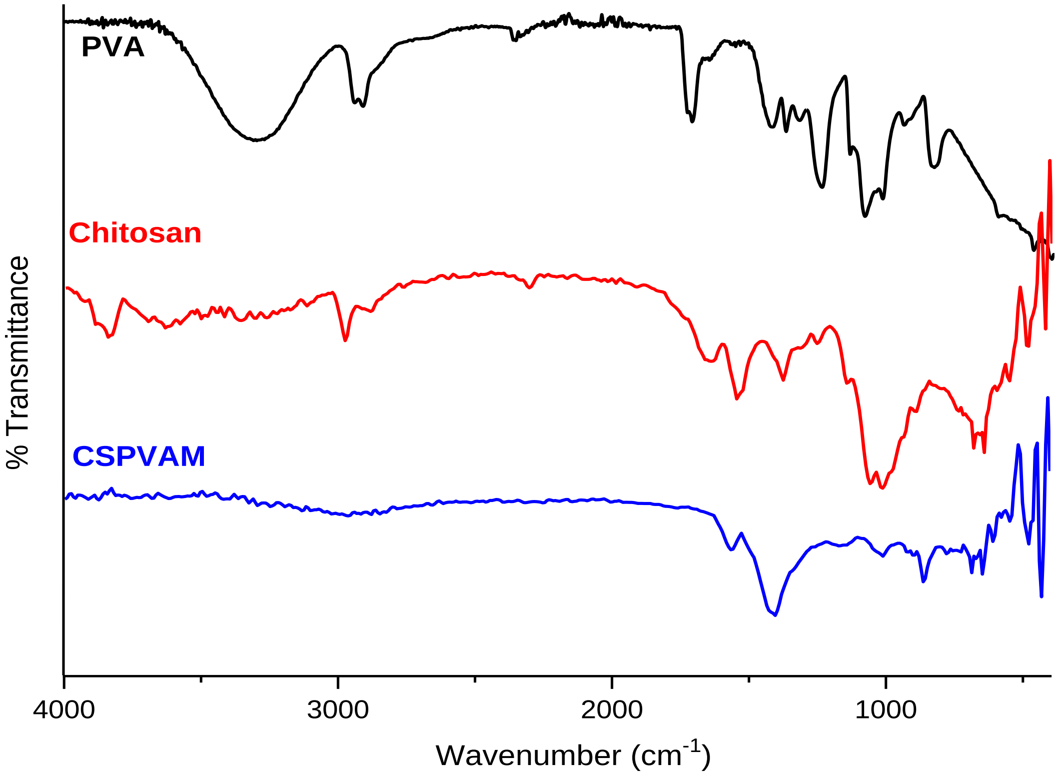

Fig 4.1: FTIR Spectra of PVA, Chitosan and CSPVAM Hydrogel

The FTIR spectra of raw chitosan and grafted chitosan generated under ideal circumstances. The spectrum shows the main characteristic peak of chitosan. The overlapping of –OH and $-NH_2$ stretching vibrations causes the absorption peak at 3283cm^{-1}.Other absorption peaks are seen at 2875, 1647, 1371, and 1024 are attributed due to the N-H bending vibrations, C-N stretching vibrations and C-N stretching and C-O stretching.

The CSPVAM hydrogels showed only less intense peak at 4000–1700 cm^{-1} which indicated the involvement of free NH and OH ends in cross linking **(C. Radhakumary et. al, 2006)**. The FTIR spectrum shows a new peak at 1660 cm^{-1} due to the C=O of grafted PVA on chitosan **(Dong Kweon et. al, 1999).** The carbonyl of the amide group showed its stretching in the region of 1635-1500 cm^{-1}. The band appears at 1655 cm^{-1}, which is demonstrative of the (─CON─) carboxyl amide group vibration of the methionine moiety attached intothe chitosan. Moreover, the intensity of the band at 1385 cm^{-1}increases due to the incorporation of additional amine groups to the CS base **(Hajipour et. al, 2016).** The changes observed in the FTIR spectrum of CSPVAM conformed cross linking of Chitosan and PVAM.

4.3. Powder X-ray diffraction

P-XRD is widely used for phase identification of a crystalline material. The XRD patterns of pure Chitosan and CSPVAM were recorded and shown in Figure7. The X-ray diffraction studies of pure chitosan exhibit very broad peaks at 2θ = 20 **(Kumar, S., & Koh, J., 2012).** The X- ray diffraction pattern of CSPVAM is showed sharp peaks at 2θ values 30.91°, 33.08°, 34.23°, 37.01°, 38.84°, 42.25°, 46.04°, 57.82°, 72.57°which were due to crosslinking of PVAM to the chitosan.

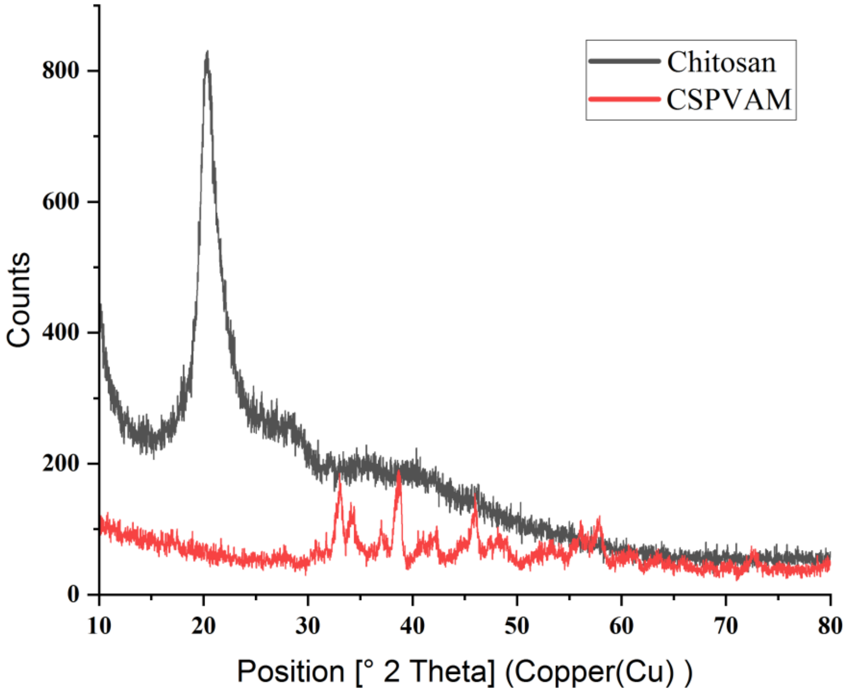

Fig 4.2: XRD of Chitosan and CSPVAM Hydrogel

The XRD patterns indicate that the chitosan contain also an amorphous form due to the presence of OH and NH_2 groups which form the intermolecular hydrogen bond. Thus, this distribution will have some regularity to build easily the crystalline regions, similar to the reported chitosan binary blend, the significant shift in the diffraction peaks and the diffraction pattern with a broad amorphous peak indicated that there was a molecular miscibility and interaction between the components **(Ramya et. al, 2012)**. The Debye-Scherrer method was used to obtain the grains' size from X-ray diffraction measurements by following relationship.

$$D=k\lambda B\cos(\theta)$$

Where D is the grain's size diameter

B is the broadening of the diffraction line measured at half-maximum intensity

λ is the wavelength of (Cu Kα)

θ is the Bragg angle for a given diffraction and k is a constant (in general equal to 0.9 for powders) **(Kumar, S., & Koh, J., 2012).**

Table 4.1: X-Ray Difrraction values

Polymer	**Position (2θ)**	**FWHM (2θ)**	**d-spacing(Å)**	**Crystalline size (nm)**
Pure Chitosan	20.3115	0.8160	4.36864	100.00
CSPVAM	30.9117	0.9368	2.89287	14.11
	33.0833	0.2676	2.70777	91.41
	34.2303	0.5353	2.61962	56.29
	37.0129	0.2676	2.42883	41.76
	38.8411	0.5353	2.31861	100.00
	42.2519	0.5353	2.13900	22.08
	46.0498	0.2676	1.97105	48.37

The default value of wavelength of X- ray diffraction is set set as 0.15418(Cu K-alpha) which is mostly used in the instruments. The crystalline size of CSPVAM was calculated by using XRD Crystallite (grain) Size calculator (Scherrer Equation)- InstaNANO. https://instanano.com/characterization/calculator/xrd/crystallite-size/.

4.4. Thermogravimetry and Differential Thermal Analysis

TGA has been extensively used to determine the thermal stability of the compounds and its volatile components by monitoring the weight loss as a function of temperature.The thermogravimetry of the CSPVAM was studied and compared with that of Chitosan shown in Fig 4.3 and 4.4

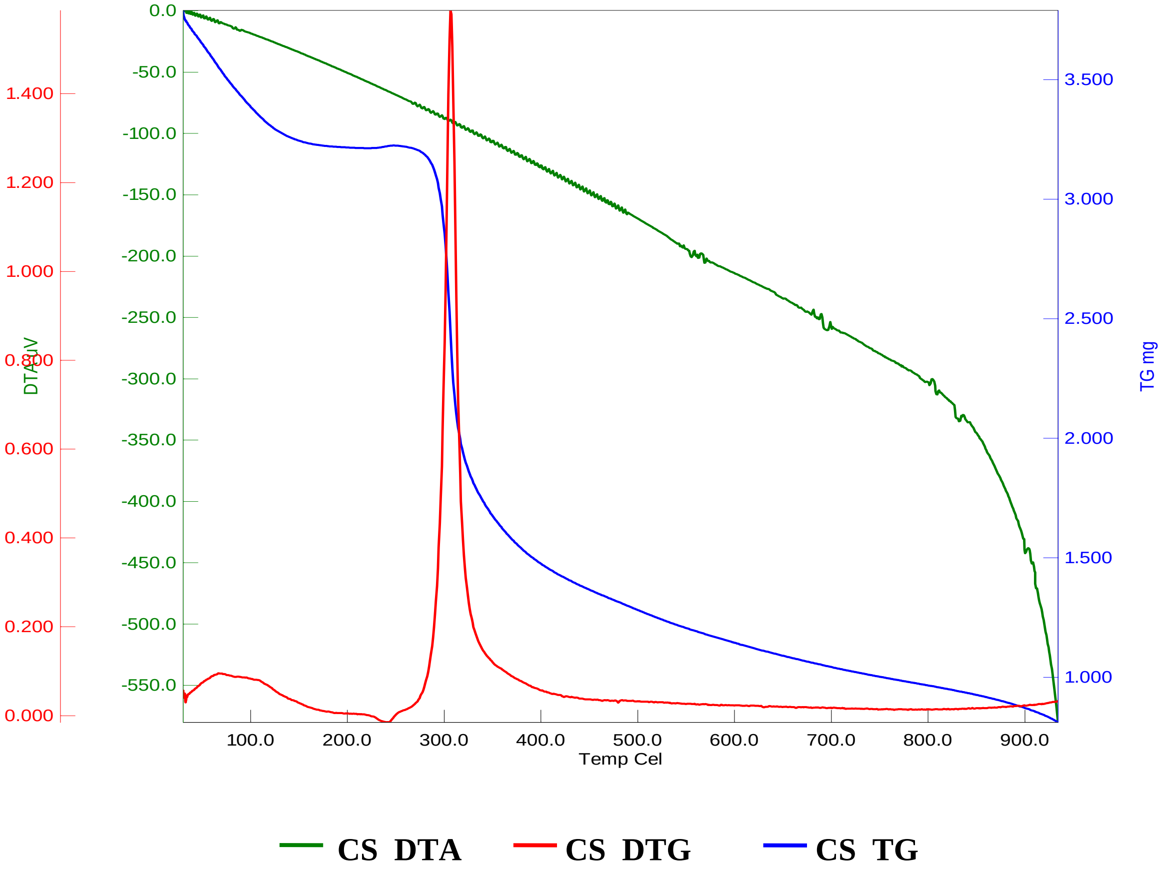

Figure 4.3: DTA, DTG and TG of CS

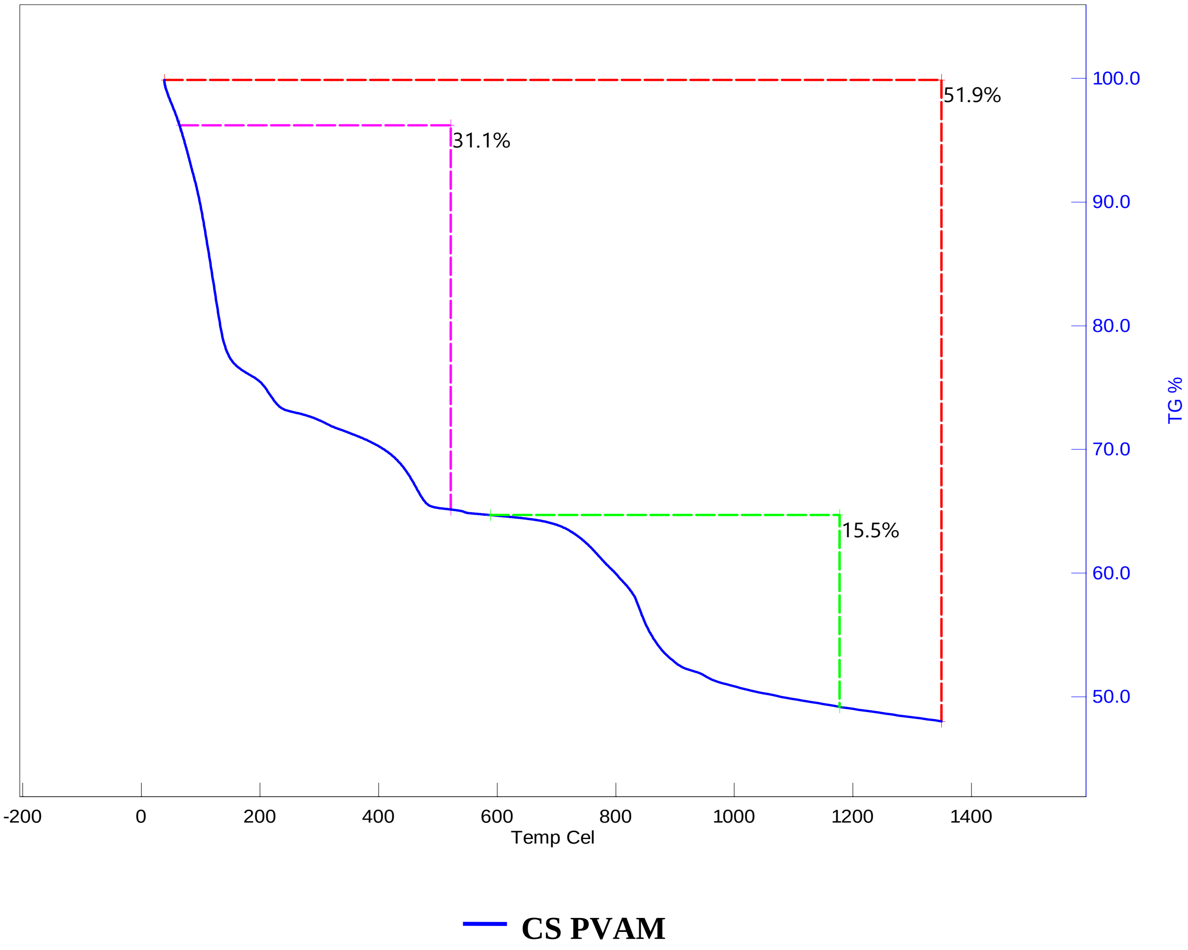

Fig 4.4: TG of CSPVAM

The TGA curve of pure chitosan shows that the two stages of weight loss is in the range from 100 to 900 °C, the first occurring in the range of 100–200 °C due to loss of water molecules. The primary degradation of pure chitosan started at 247 °C and it was completely degraded at about 900 °C.TGA of CSPVAM showed two different stages of weight loss. The first stage of weight loss, starting from 100 to 500°C, may correspond to the loss of adsorbed water. The second decomposition stage occurs in the range 580 to 1180°C. The results demonstrate the loss of the thermal stability for CSPVAM compared to the Chitosan. The thermal analysis showed that the CSPVAM are stable from. Thermal stability of CSPVAM was confirmed by TGA analysis showing no weight loss from 1180 to 1400^{o} C since there is no weight loss.

4.5. Swelling studies in different salt solutions

CSPVAM hydrogels were studied for their water absorbency in various salt solutions. The hydrogels were dipped into the different concentrations of the salt solutions at room temperature for 3 hours. The amount of water absorbance in CSPVAM hydrogel have be calculated using the equation 2 and showed in Fig 4.5

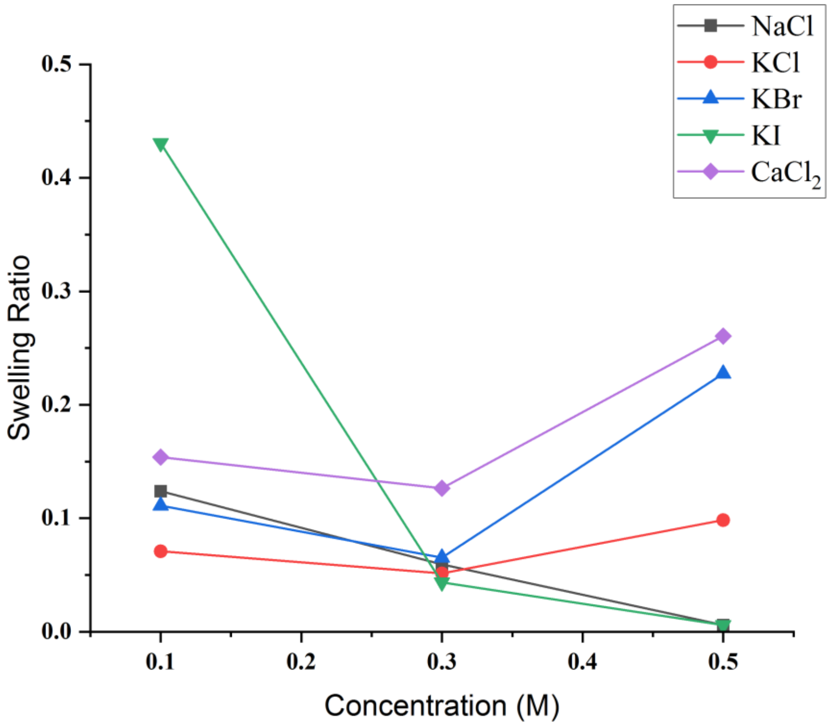

Fig 4.5: Swelling behavior of CSPVAM

The swelling medium affects the absorbency behavior of hydrogels because of the change in the environmental ionic strength. The swelling ratio of the hydrogel is decreased in the presence of NaCl, KBr, KI and $CaCl_2$. In salt solutions, the swelling capacity decreases because of the ions' screening effect and the differential between the internal osmotic pressure of mobile ions both inside and outside the gel **(Sadeghi et. al, 2012)**. This is explained in detail by Equilibrium theory of Donnan. When the swelling is balanced, the water's chemical potential inside the polymer network and outside the polymer is same as water. When a simple salt is added to a polymer solution, the polymer network contracts, lowering the chemical potential of the water surrounding the polymer. As a result, absorbent polymers are unable to absorb as much salt water **(Geethanjali**

et. al, 2008). Swelling is related to the concentration of the salt solution as, **(Gupta, N. V., & Shivakumar, H. G , 2012)**.

$$\text{Swelling} = k\,[\text{salt}]^{-n}$$

Here, k and n are constants for an individual super porous hydrogel. The k value gives the swelling at a high concentration of salt and the n value is a measure of salt sensitivity. So, at higher concentration of the salt solution, the swelling ratio increased for KBr, KI and $CaCl_2$.

4.6. Swelling Behavior in physiological solutions

Different biological solutions (physiological saline water, synthetic urine, urea solution, D-glucose solution) were prepared as described in table 2. The swelling behavior of the hydrogels at different time intervals were studied for 3,12and 24 hrs and are depicted in Fig 4.6. The graph shows the increased swelling ratio with increase in the immersion time for urea and glucose.

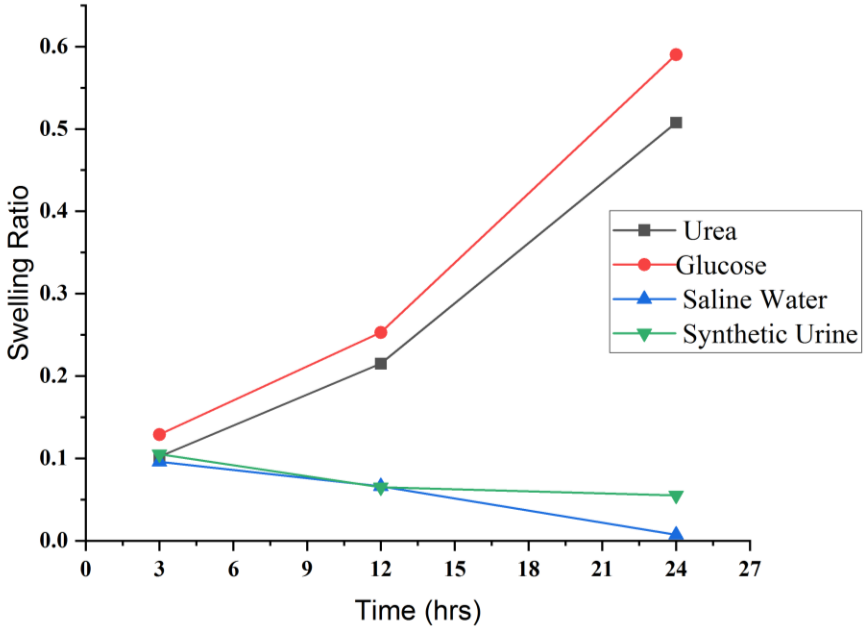

Figure 4.6: Swelling behavior of CSPVAM in physiological solutions

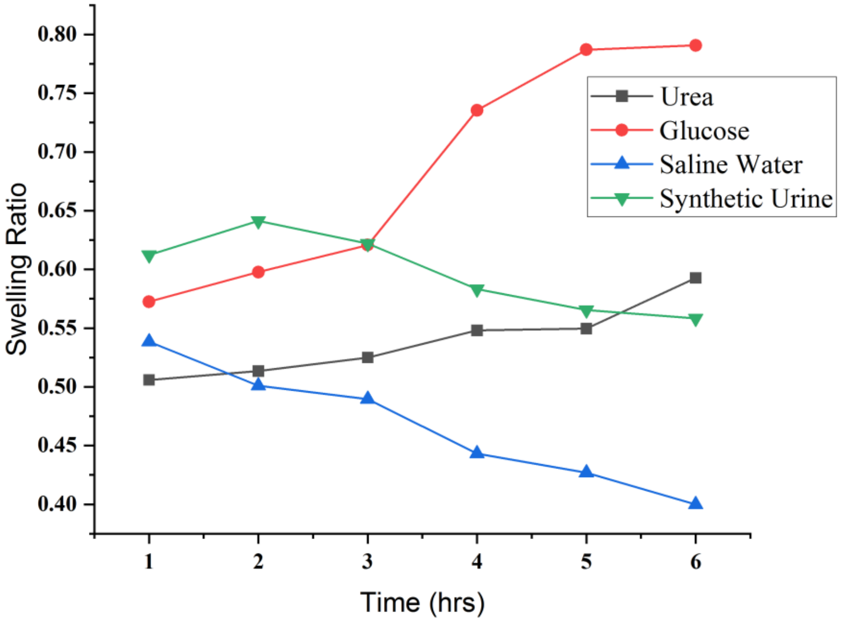

Fig 4.7: Time studies for swelling behavior of CSPVAM in physiological solutions

The order of the swelling ratio of the samples in different biological fluids is as follows (in Fig 4.7): urea solution > glucose solution > saline water ≈ synthetic urine. The swelling ratios of the sample in urea solution and D-glucose solution are higher than those of the corresponding swelling in other salt solutions. The urea and glucose molecules can penetrate into the hydrogels thereby increasing its swelling ratio.

The fluid absorbencies of the CSPVAM in Saline and Synthetic urine solution were decreased as the time period increases due the presence of NaCl. The osmotic pressure promoting water penetration into the hydrogel structure reduces, thereby resulting in a reduction in swelling. In general, the swelling ratio of CSPVAM hydrogel is determined by the ionic group's association state within the polymer and the complex's affinity for water. As per the Donnan osmotic pressure equilibrium, increase in the moveable counter ions of a solution causes a drop in the osmotic pressure within the hydrogel, leading the hydrogel to shrink**. (Ying Zhao et. al, 2006 and Kim et. al, 2005).**

4.7. Swelling Kinetics in Water

At different time intervals, the swelling capacity of the CSPVAM in distilled water was studied and depicted in Fig 4.8. It clearly showed increase in the rate of water intake initially which tends to achieve an optimum state as time proceeds.

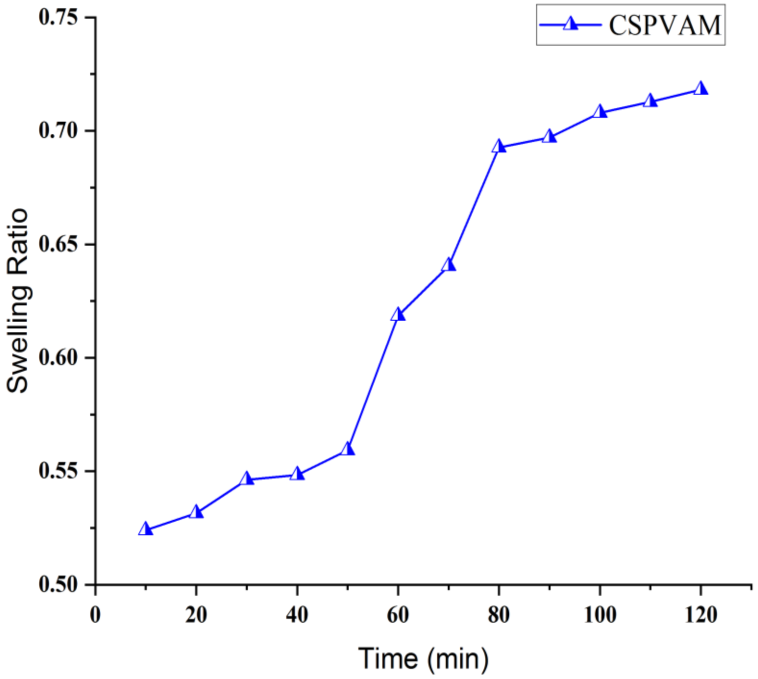

Fig 4.8: Swelling studies of CSPVAM in different interval of time

The affinity of polymeric hydrogels for solvents can be easily determined by their sorption ability. The swelling process of polymers involves the diffusion of the liquid phase into the hydrogel bulk, which is possible by the mobility of the polymeric chains and the free-volume among chains. The molecules of the liquid remain entrapped in these free spaces up to an equilibrium point, as determined by both polymer solvent interactions and the elasticity of the hydrogel **(Farag et. al, 2012).**

The equilibrium swelling was achieved after 100 min. The swelling corresponds to the crosslinking behavior. In this study, the prepared CSPVAM hydrogel showed a maximum water-swelling ratio of about 0.70 which 70% of its weight. A power law behavior is obvious from Fig 4.8. The data may be well fitted with a Voigt-based equation 3, **(Omidian, H. et. al, 1998).**

St = Se(1 –e-t \ד) (3)

where St (g/g) is swelling ratio at time t, Se is equilibrium swelling ratio (power parameter, g/g), t is time in minutes for swelling and ד in minute stands for the rate parameter. To calculate the rate parameter, by using the above formula the equation 3 can be rearranged and plotted for ln(1 -St\Se) versus time (t), as shown in Fig 4.9.

Table 4.2: Rate Parameter by Voigt-based equation

Time(min)	**St**	**Se**	**St/ Se**	**1- St/ Se**	**ln(1- St/ Se)**
10	0.524	0.708	0.7401129944	0.2598870056	-1.347508336
20	0.5315	0.708	0.7507062147	0.2492937853	-1.389123217
30	0.5463	0.708	0.7716101695	0.2283898305	-1.476701327
40	0.5484	0.708	0.7745762712	0.2254237288	-1.489773409
50	0.5592	0.708	0.7898305085	0.2101694915	-1.559840971
60	0.6185	0.708	0.8735875706	0.1264124294	-2.068205468
70	0.6405	0.708	0.9046610169	0.09533898305	-2.350316496
80	0.6926	0.708	0.9782485876	0.02175141243	-3.828076584
90	0.697	0.708	0.9844632768	0.01553672316	-4.164548821

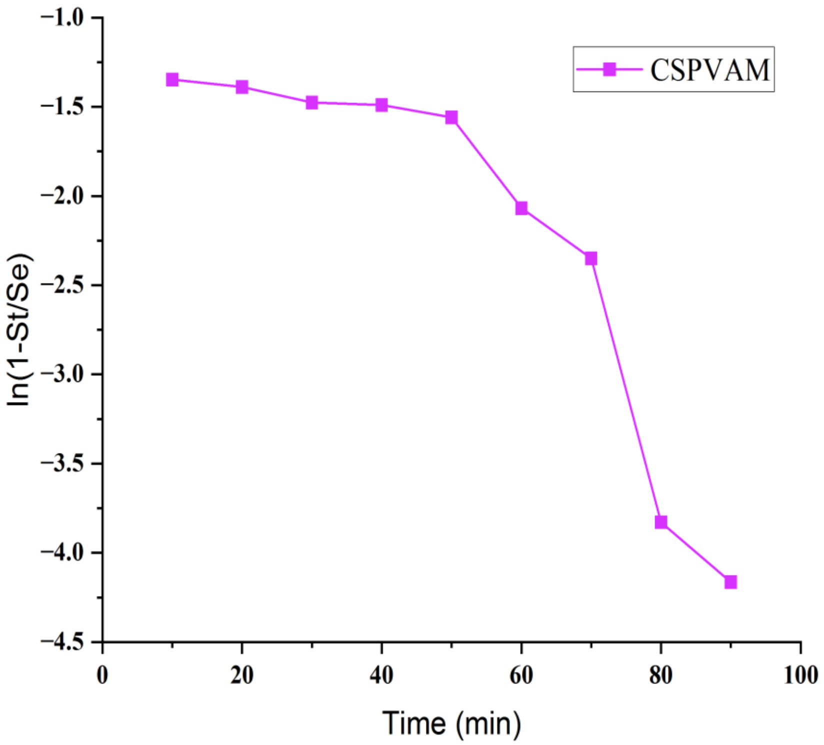

Fig 4.9: Swelling kinetic - Voigtplot for hydrogel

The slope of the fitted straight line (slope = - 1\ ד) gives the rate parameter. According to the fig 4.9 and using equation (3), the rate parameter for the hydrogel in water is found to be 36.69 minutes. Since the ד value is a measure of swelling rate, the lower the ד value, the higher the rate of swelling.

4.8. Swelling studies at different pH

The equilibrium swelling kinetics of CSPVAM Hydrogel at different pH 3, 4, 7, 8 and 10 were studied at room temperature for 3 hrs immersion time and are presented in Fig 4.10.

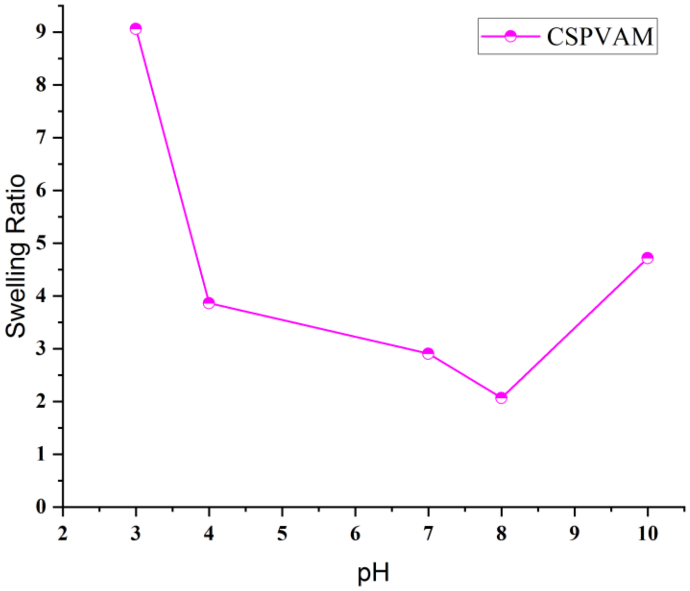

Fig 4.10: Swelling ratio of CSPVAM at different pH level

The swelling ratios of the hydrogel in different pH buffer solutions are varied with pH of the medium. The figure depicts that the hydrogel swelled more at pH 3 and shrank at pH 8. At pH 3, CSPVAM had the highest swelling ratio of the samples because it included the most chitosan. Because of the repulsive attraction between similar charges of molecules, positively charged chitosan at a low pH demonstrated a high swelling ratio, resulting in a long intermolecular distance and a more hydrophilic state, both of which contribute to network growth. The swelling of a hydrogel with a high concentration of a charged ionic group is known to grow due to osmosis and charge repulsion. Alkaline hydrogen bonds, on the other hand, tend to associate because the $–NH_3$ groups can be converted to $–NH_2$ groups due to the low H concentration. The protonation of amino groups ($–NH_2$) in chitosan hydrogel and the breakdown of hydrogen bonds, which causes hydrogel swelling, provide an internal ion osmotic pressure in an acidic solution **(Kim et. al, 2005).**

This figure showed the shrinking of the hydrogel at pH 8. The hydrogels exhibit high sensitivity to pH change which is the promising attribute for the adoption of the hydrogels in the biosensor applications.

5. SUMMARY AND CONCLUSION

Today hydrogels find a wide range of application because of their lower cost, non toxic, and environment friendly nature. The most important characteristics of the hydrogels to function as an absorbent for aqueous fluids are hydrophilic nature and swelling behaviour of the polymer. Hydrogels have been used for widespread applications in different fields, such as agriculture and horticulture, removal of dyes and metal ions from water, medical, pharmacy and biotechnology etc

The current work on **Synthesis of Chitosan Polyvinyl Alcohol Methionine Hydrogel and its Swelling Responses in Physiological fluids** is summarized as follows,

- ✓ The Chitosan has been selected as a polymeric backbone to synthesise the hydrogels. PVA is first grafted with L-methionine using ammonium persulphate in oxalic acid medium at 0-5 ^{o}C. The formed grafted PVA (PVAM) is precipitated out using acetone. PVAM is dried and used for crosslinking with chitosan. The grafted polymer, PVAM is cross linked with chitosan using N,N'-methylene bisacrylamide and ammonium persulphate to form Chitosan polyvinyl alcohol methionine – N,N'-methylene bisacrylamide CSPVAM hydrogels.
- ✓ The dried samples of CSPVAM were characterized using a FTIR spectroscopy, and Powder X-Ray Diffraction Techniques. The thermal stability of the g-polymer is tested using Thermogravimetry and Differential thermal analysis.
- ✓ The amorphous form of CSPVAM were confirmed by PXRD results. The amorphous size of the hydrogel were calculated using Scherrer equation.
- ✓ Thermal stability of CSPVAM was confirmed by TGA analysis showing no weight loss from 1180 to 1400 ° C.

The swelling response of the synthesized CSPVAM hydrogels were studied

- In Different Salt Solution (NaCl, KCl, KBr, KI and $CaCl_2$)
- In Physiological Solutions (Urea, Glucose, Saline Water and Synthetic Urine)
- In Distilled water for various immersion time
- In Distilled water for 3 hrs in different pH

In salt solutions, the swelling capacity decreases because of the ions' screening effect and the differential between the internal osmotic pressure of mobile ions both inside and outside the gel. As a result, absorbent polymers are unable to absorb as much salt water, So the swelling ratio increased for KBr, KI and $CaCl_2$.

In Physiological Solutions the order of the swelling ratio of the samples in different biological fluids is as follows

Urea solution > glucose solution > saline water ≈ synthetic urine.

The swelling ratios of the sample in urea solution and D-glucose solution are higher than those of the corresponding swelling in other salt solutions. The fluid absorbencies of the CSPVAM in Saline and Synthetic urine solution were decreased as the time period increases due the presence of NaCl. The osmotic pressure promoting water penetration into the hydrogel structure reduces, thereby resulting in a reduction in swelling

In distilled water the equilibrium swelling was achieved after 100 min. The swelling corresponds to the crosslinking behaviour of the hydrogels. In this study, the prepared CSPVAM hydrogel showed a maximum water-swelling ratio of about 0.71 which is 71% of its weight.

The swelling ratios of the hydrogel in different pH buffer solutions are varied with pH of the medium. The hydrogel swelled more at pH 3 because the protonation of amino groups ($–NH_2$) in chitosan hydrogel and the breakdown of hydrogen bonds, which causes hydrogel swelling, provide an internal ion osmotic pressure in an acidic solution and showed the shrinking of the hydrogel at pH 8. These ionizable groups dissociate or protonate depending upon the pH of the swelling medium.

Hence these CSPVAM Hydrogels can be suggested in the field of Agriculture as a slow-release fertilizer (Urea) or in the biomedical or biosensors applications like glucose sensors, target – oriented drug delivery and so on.

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