"SYNTHESIS OF BIODEGRADABLE HYDROGEL-BENZIPORPHODIMETHENE BASED METAL SENSOR"

A Major Project Report Submitted in the partial fulfillment for the Award of the degree of

MASTER OF TECHNOLOGY

In

POLYMER TECHNOLOGY

Submitted by

SUSHMA

2K14/PTE/10

Under the Supervision

Dr. Anil Kumar & Mr. S. G. Warkar

Assistant Professor

Department of Applied Chemistry

& Polymer Technology

Delhi Technological University

New Delhi-110042

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DECLARATION

I SUSHMA hereby declares that the thesis entitled **"SYNTHESIS OF BIODEGRADABLE HYDROGEL-BENZIPORPHODIMETHENE BASED METAL SENSOR"** is an authentic record of research work done by me under the supervision of **Dr. Anil Kumar and Mr. S. G. Warkar,** Assistant professors Delhi Technological University, Delhi. This work has not been previously submitted for the award of any degree or diploma of this or any other University/Institute.

SUSHMA

M.Tech (Polymer Technology)

(2K14/PTE/10)

CERTIFICATE

This is to certify that the project entitled **"SYNTHESIS OF BIODEGRADABLE HYDROGEL-BENZIPORPHODIMETHENE BASED METAL SENSOR"** submitted by SUSHMA (2K14/PTE/10) in partial fulfillment for the award of degree of master of technology in polymer technology to Delhi Technological University, Delhi, is recorded of the work carried out by him under our supervision. The project embodies the original work done by him to the best of our knowledge and has not been submitted to any other degree of this or any other university. The matter embodied in this project is original and not copied from any source without proper citation.

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ACKNOWLEDGEMENT

I am heartily thankful to my supervisor, **Dr. Anil Kumar** and **Mr. S. G. Warkar** for taking out their valuable time and patiently familiarizing me with the fundamentals, for teaching with complete passion and energy and helping me completely.

I am pleased to acknowledge, **Professor R. C. Sharma (** Head of Department **)**, for providing me with an opportunity to complete my project at the Department of Applied Chemistry and Polymer Technology and without whom encouragement, this project would not have been successful.

I am also grateful to Mr. Ravi Kumar Sharma and Mr. Om Prakash, Ms. Nidhi who helped me clear doubts whenever they arose and helped me substantially for my project.

Finally, I would like to thank the faculty and the staff of the Department of Applied Chemistry and Polymer Technology for providing me with an environment where I was able to contribute and accomplish tasks equal to my interest and knowledge.

ABSTRACT

Biodegradable hydrogel based on polyacrylamide/carboxymethylguargum were synthesized by in situ incorporation of fluorescent molecule Benziporphodimethenes and potassium persulphate as an initiator and N'N methylbisacrylamide as cross-linking agent for detection of Zn^{2+} ion in aqueous solution. The free molecule in hydrogel is almost optically transparent. The modified hydrogel shows a colorimetric response to Zn^{2+} ions in aqueous solution in the presence of other metal ions whose aqueous solution is colorless.

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Chapter 1

INTRODUCTION:-

Metal ions play a most important role in biological processes in organism. The detection of heavy metal ions is important in many applications, including environmental monitoring, waste management, developmental biology, and clinical toxicology [1]. Heavy metals, such as Cd, Pd and Hg are the most hazardous, because of their high solubility in the aquatic environments. They can be absorbed by living organisms, aggregate in the human body, and cause serious health impacts as cancer, organ damage, nervous system damage, and sometimes death. There are a lot of techniques known in the literature to remove such heavy metal ions. These techniques include filtration, chemical precipitation, chelating ion-exchange, and adsorption. The disadvantages of these techniques are due to their cost, environmental and health problems related to low efficiency, long-time processing and high-energy consumption. However, adsorption is an attractive method because of its high efficiency, easy operation, low cost and availability of different adsorbents [2-3].

Most common sources of heavy metals to waste water are mining and extraction, waste disposal, agriculture and metallurgical industries. The important question comes in the human mind why heavy metal ion detection is important? Usually, heavy metals are earth's crusts natural constituents. Buthuman activities have drastically changed their biochemical balance and geochemical cycles. This results in accumulation of metals in plant. Lengthened exposure to heavy metals such as copper, cadmium, lead, zinc, mercury and nickel can cause harmful health effects in humans [4].

Heavy Metals and Living Organism

Living organisms require trace amounts of heavy metals like manganese, iron, cobalt, copper, and zinc. All metals are toxic at higher concentrations and can cause serious health issues to the organism. Other heavy metals like mercury, plutonium, and lead are toxic metals that have no known vital effect on organisms, but their accumulation over time in the bodies of animals can cause serious illness [5].

Table 1: Heavy metals and their effect on human health and their permissible limits

Metal	Sources	Effect on human health	Permissible limit(mg/l)
Zinc	Refineries, metal plating, brass manufacturing,	Zinc fume have corrosive effect on skin, cause damage to nervous membrane	15
Lead	Paint, pesticide, smoking, mining, burning of coal	Mental retardation in children, liver, kidney, gastrointestinal damage	0.1
Chromium	Mines, mineral sources	Damage to nervous system, fatigue, irritability	0.05
Arsenic	Pesticides, metal smelters, fungicides	Bronchitis, poisoning, dermatitis	0.02
Manganese	Welding, ferromanganese production	Inhalation or contact cause damage to central nervous system	0.26
Copper	Mining, metal piping, chemical industry, pesticide production	Anemia, liver and kidney damage , stomach and intestinal irritation	0.1

Heavy Metals and Environmental pollution

Metal concentration in soil typically ranges from 1 to 100,000 mg/kg. Heavy metals are the main group of inorganic contaminants because use of municipal compost, fertilizers, pesticides, and emissions from municipal wastes incinerates residues from mines, exudates, and smelting industries. Excessive levels of heavy metals result degradation of soil quality, poor quality of agricultural products, crop yield reduction and posing significant hazards to animal, human, and ecosystem health. Therefore remove of the accumulated metals becomes essential [5].

Heavy Metal and Polymer

Separation and removal of metal ions has attractive areas of research and led to new technological developments. Ion exchange polymers and metal chelating were used in hydrometallurgical applications like removal of traces of radioactive metal ions from wastes and recovery of rare metal ions from seawater. A polymeric material is used for selectively binding a specific metal ion form a mixture to isolate important metal ions from aqueous media and wastewater. An insoluble resin used to separate a specific metal ion from a liquid or aqueous solution containing a mixture of metal ions [5]. Polyvinyl alcohol hydrogel based fiber interferometer used as heavy metal cations sensor. Fluorescent carbon quantum dot hydrogels used for detection of silver ions [6]. Poly (acrylamidoglycolic acid-coacrylamide) hydrogel for selective binding of Cu^{2+} [7].

The use of heavy metals by modern industries has led to increase environmental problem. These heavy metals (Hg, Pb, Ni, Cu, Cd, Zn) are non biodegradable, accumulate in the ecological systems and posing a serious threat to human because of its toxicological and carcinogenic effects. Because of the known harmful effects on human health there is active research is developed for trace of heavy metals and various health and standards of maximum allowable heavy metals [5].

Table 2: Heavy metals and their effect on human health and their permissible limits

Separation techniques	Advantage	Disadvantage
Adsorption	Economical and efficient for aqueous solution	Regeneration needed
Membrane separation	Excellent heavy metal removal	Membrane fouling cost
Ion exchange	Easy removal of heavy metal ions	Fouling and regeneration

Chemical precipitation	Remove most heavy metal ions	Need to adjust P_H and break down the complex
Electro dialysis	Remove most ions with electric charge	Unsatisfactory for removal of chelated ions

Current techniques require costly and sophisticated equipment for trace heavy metal detection like inductively coupled plasma atomic emission spectroscopy and atomic absorption spectroscopy. These are operated by highly trained personnel and remote monitoring applications. Therefore we used polymeric hydrogel for detection of heavy metals. Hydrogels are hydrophilic polymer networks that are capable of absorbing large amounts of water. For this reason they have been used widely in the field of drug delivery, dewatering of protein solutions, solute separation, baby diapers, soil for agriculture, water-blocking tapes, absorbent and numerous other applications. However, they are insoluble because the hydrogel chains are joined covalently in the form of three-dimensional networks. High swelling rate is an important property of hydrogels. They have been used for removing heavy metal ions from wastewater due to three-dimensional network structures and their ability of joining different functional groups. These ionisable groups, such as carboxylic when ionized, produce fixed ions that repel one another, and this repulsion leads to greatly enhanced swelling of the network. The binding and capturing is the most important application of hydrogel [9].

What is Gel

Gel is defined as a solid jelly like soft material. Its property ranging from soft and weak to hard and tough. Gel has both solid and liquid component, solid component present as a network of aggregates, which immobilizes the liquid component. Gels by weight are mostly liquid, but they behave as solids because of their three dimensional cross-linked network within the liquid [10].

We can also say that gels are dispersion of liquid molecules in solid cross lined network in which liquid is a discontinuous phase and solid is a continuous phase. Because of this solid network gel prevents the flowing of liquid by increasing the surface tension. The bonds in the gel may be physical bond, chemical bond and crystallites also.

Gels are generally two types

- Organogel
- Hydrogel

Organogel

Organogel is defined as a thermoplastic, non–crystalline solid material composed of a liquid organic phase in a three-dimensional cross-linked network. The liquid can be a vegetable oil, organic solvent, or mineral oil [10].

Hydrogel

Hydrogel is defined as a transparent, water swell able, viscoelastic and thermo dynamically stable, three dimensional polymeric networks. They contain water soluble groups such as –COOH, -OH, -$CONH_2$, -NH_2, and-SO_3H [1]. They have high capacity to absorb water it can be 1000 time of weight of polymer. Because of its combined glassy and elastic behavior they have many applications and are highly studied. Hydrogels have been used in the fields of biotechnology, medicine, pharmacy, food industry, agriculture etc. Hydrogels are three dimensional cross-linked hydrophilic polymers that swell in water and aqueous solutions without dissolving in them. Softness, smartness, and the capacity to store water make hydrogels unique materials. Several techniques have been reported for the synthesis of hydrogels like co-polymerization/crosslinking of co-monomers using multifunctional co-monomer, which acts as crosslinking agent. They can be classified in different ways on the basis of their preparation, biodegradable properties, polymer, and sensitivity to surrounding environment and also their application. Hydrogels being biocompatible materials have been recognized to function as drug protectors, especially for peptides and proteins, from in-vivo environment. Hydrogels that are responsive to specific molecules, such as glucose or antigens, can be used as biosensors as well as drug delivery systems. Hydrogels can be prepared from natural and synthetic materials [12].

Classification based on source

1. Natural
2. Synthetic .

Classification according to polymeric composition

Depend on polymeric composition hydrogel classified in three types:

(a) **Homopolymeric hydrogels** are referred to polymer network formed from a single monomer, which is a basic unit forming a polymer network. Homo polymers have cross-linked structure depending on the polymerization technique and nature of the monomer

(b) **Copolymeric hydrogels** are referred as polymeric network of two or more different monomer species in which at least one is hydrophilic in nature. The monomers can arrange in a random, block and alternating form along the chain of the polymer network.

(c) **Multipolymer Interpenetrating polymeric hydrogel** (IPN)
Animportant class of hydrogels, is made of two independent cross-linked synthetic and/or natural polymer component, contained in a network form. In semi- IPN hydrogel, one component is a non-cross-linked polymer and other component is a cross-linked polymer [10].

Depending on pore size they are classified as:

Type	Morphology	Major swelling mechanism	Swelling rate	Application
Non-porous	Without network porosity	Diffusion through free volumes	Very slow, sample size-dependent	Contact lenses, artificial muscles, Etc.

Micro-porous	Various porosity with closed-cell structure (100-1000 A)	Combination of molecular diffusion and convection in the water filled pores	Slow, sample size-dependent	Mainly in biomedical applications and controlled release technology
Macro-porous	Various porosity with closed-cell structure (0.1-1μm)	Diffusion in the water filled pores	Fast, sample size-dependent	Mainly in form of superabsorbent in baby diapers, etc.
Super-porous	High porosity with interconnected opencellstructure	Capillary forces	Very fast, sample size-dependent	Drug delivery (particularly in the gastrointestinal tract), tissue engineering, etc.

Table 3: Types of hydrogel on the basis of pour size.

Why we used polyacrylamide based hydrogel:

We used polyacrylamide based hydrogel because it is most suitable for optical studies because it remains optically transparent for a wide range of concentrations of the cross-linker and the monomer.It is an atoxic, stable hydrogel. Polyacrylamides based cross linked hydrogels are soft gels, highly water-absorbent used in following applications like electrophoresis and in manufacturing soft contact lenses. It is also used as a thickener and suspending agent in straight chain form. Polyacrylamide based hydrogels are widely used for removal of heavy metal ions from water system and protect the human being and the environment from harmful effect caused by heavy metal. Fluorescent molecules for the recognition of metal ions are of great interest for chemist as well as biologist because of their significant role in biological and environmental process [12-13].

SCOPE OF THE WORK

Based on the available literature, no colorimetric sensor is available to sense the zinc metal ion in water based on polymeric hydrogel. Interestingly, zinc is the spectroscopically silent metal ion because of d^{10} system. Its metal salts are colorless in water so it becomes very difficult to sense zinc ion in solution.

In the present work, we have tried to synthesize biodegradable hydrogel-Benziporphodimethene based metal sensor, for metal ion detection. These modified hydrogels were characterized using available characterization techniques.

Chapter 2

LITERATURE REVIEW:-

We know that heavy metals are harmful and toxic in excess to human being and environment therefore it is important to detect and remove metal ion from aqueous solution. There are so many techniques are already used for removal of metal ion from aqueous solution like adsorption, ion exchange, precipitation and electro dialysis etc. In all these technique adsorption is the most frequently used for purification? They bind and capture metal ion from aqueous solution for removal of heavy metals from dilute solution. The important part of adsorption is adsorbents. Therefore good adsorbent should be abundant and easy to process and have high selectivity, long service time and large surface area [2-3].

Some natural and synthetic compounds used as adsorbent are clay, zeolite, peat, bark, lignin, dead biomass wool, fly ash and cotton, chitosan as well as polymer, copolymer, composite etc [3].

Compared to above adsorbent hydrogel based adsorbent are recently show special significant attention for removal of metal ion from aqueous solution. They have chemically-responsive functional group and three dimensional porous structures which are important for capture and binding the ion from aqueous solution [2] Johnet. al. prepared a poly (acrylamidoglycolic acid-co-acrylamide) hydrogel for choosy binding of Cu^{2+}. They used this hydrogel for the application of diffusive gradients in thin films measurements. They prepared the hydrogel by copolymerizing 2-acrylamidoglycolic acid (AAGA) and acrylamide (AAm) with 7:2 ratios of monomers respectively [6]. The hydrogels metal ion binding property were characterized by a range of metal ions (Cu^{2+}, Cd^{2+}, K^{+}, Na^{+}, Mg^{2+} and Ca^{2+}) with changeable conditions of pH, metal concentration, ionic strength and time. The formed hydrogel show $Cu^{2+}$and Cd^{2+}binding in non-competitive binding condition. The capacity of binding of each metal ion decreased, in competitive binding conditions. The capacities of binding for Cu^{2+} and Cd^{2+}were also decreased with increasing ionic strength and at pH values less than 5 [6].

Later Mahlousand his coworkers developed a type of acrylamide - Co - acrylic acid hydrogel synthesized and crosslinked by gamma radiation for adsorption of metal Ions and dyes. The swelling property test is performed on Congo red and methyl violet dye solution and water.

This test show that swelling rate depend on ionic nature and pH of dyes. The swelling actions and ability to absorb dyes by the hydrogel seems to be higher at basic medium. That Cu^{2+} metal ion adsorption increase on pH increasing from pH1 to pH4. The repulsion forces between anions makes the polymer structure more "open" for the diffusion of water molecules while at lower pH, the protonation of carboxyl groups restrict the diffusion of water molecules. Alternatively, at higher pH, due to the delocalization of the charge between oxygen atoms, they may bond more water molecules [15].

Recently Zheng used hydrogel for detection and removal of heavy metal ions from aqueous solution. In this review paper they developed three type of hydrogel for removal of heavy metal ion and discussed associated ion capture-released mechanism. Three type of hydrogel used are: protein or DNA functionalized hydrogel, temperature swing hydrogel and crown ether functionalized hydrogel. We know that some metal ion formed specific complex with biomolecules containing nitrogen, oxygen and sulfur to introduces toxicological effect on nervous system (Hg^{2+}, Pb^{2+}, As^{3+}); the kidneys or skin, bones, or teeth (Ni^{2+}, Cu^{2+}, Cd^{2+}, Cr^{3+}) or liver (Cu^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}).They form a hybrid hydrogel using N-and C-termini of pea metallothioneins (PMTs) proteins as cross-linker. PMT binds the toxic metal ion and led to chain contraction and these metals can be removed by using chelators and can we reuse [2]. For detection and removal of Hg^{2+} from water polyacrylamide hydrogels-based sensor functionalized with a thymine rich DNA was developed. Temperature swing hydrogel composed of Poly (N-isopropylacrylamide) (PNIPAM) and a chelating or an ionic component. In which chelating compound used as capturing of heavy metals in the solution by temperature-swing adsorption. By changing the temperature the control of the adsorption and desorption of metal ions is called temperature-swing adsorption [3].

Crown ether hydrogel mostly used for detection of metal ion instead of removal of heavy metal ion. Crown ether is a synthetic host compound having ability to detect metal ions such as K^{+}, Ba^{2+}, Cs^{2+} and Pb^{2+}.When the cavity size of crown ether match with the metal ion then they are captured by crown ether receptor and forming a stable complex [3].

Chanand his coworkers devolved a Poly (vinyl alcohol) hydrogel based fiber interferometer sensor for sensing heavy metal cations like Ni^{2+}.Theincreases degree of cross linkages and increases its refractive index within the hydrogel because of presence of Ni^{2+}, leads to phase shift in the interferogram. The concentration of Ni^{2+} is monitored by the shifting of interference dips with a sensitivity of 0.214nm/ µM and limit of detection of 1nM [1].

Liu developed a new type of C2-Symmetric Benzene-based Low Molecular Weight Hydrogel tailored Electrode for extremely Sensitive Detection of Cu^{2+} ion. In this paper, they used low molecular weight gelators (LMWGs) and C2-symmetric benzene-based hydrogel (C2-BHG),in which 1, 4-dimine benzene was assembled on glassy carbon electrode (GCE) and mica surface, based on the hydrophobic interaction and hydrogen bonding. The Hydrophilicity of GCE increased by the used of C2-BHG hydrogel layer on its surface. The loaded amide and hydroxyl groups in the gel layer formulate the C2- BHG/GCE suitable as ultrasensitive sensor for Cu^{2+} ions detection by stripping voltammetry by the limit of detection of $5*10^{-10}$g/L. This new C2-BHG modified electrode was highly capable for sensing Cu^{2+} ions and used as sensor application because of its high stability and outstanding selectivity of the analytical signal [7].

Wang and his coworkers developed a hydrogel sensing film for free detection of Zn^{2+} by embedding a fluorescent indicator compound in the poly(2-hydroxyethylmethacrylate) hydrogel, the used is 11,16-bis(phenyl)-6,6,21,21-tetramethyl-m-benzi-6,21-porphodimethene [10]. The film show high stability and selectivity to Zn^{2+}. By fabricating a micron-sized pillar array, the sensitivity of the sensing film is increased because of increase of surface area of sensing film. They show the response time is 30 and 3 s for Zn^{2+}at concentrations of 10^{-4} and 10^{-3}M respectively. This sensing film is capable to be used in the integrated semiconductor optoelectronic devices as a sensing unit for Zn^{2+} sensing [9].

Chapter 3

EXPERIMENTAL WORK:-

Materials and Methods:

Acrylamide (AM) (S.D. Fine Chemicals Mumbai India), Carboxymethylated guar gum (CMG) viscosity 3000 CPS at 25^0C (Courtesy Hindustan Gum Ltd. India), N,N′- methylene bisacrylamide (N,N′MBA) (Merck, Germany), Potassium persulphate (KPS) (CDH, New Delhi India) were used as received, Acetone, Benziporphodimethene (BPDM) (florescence compound), distilled water .

Synthesis of the Hydrogel:

Take a beaker of 100ml and add 25 ml of distilled water in it. Weigh 2.5g of acryl amide and dissolved it in distilled water in beaker, put the beaker on magnetic stirrer. After that weigh 1% of potassium per sulfate by weight of acryl amide and dissolved it in beaker containing solution and added potassium per sulfate used as initiate. After that weigh 0.5% MBA by weight of acrylamide [17-18].

The mixture is stirred approximately 1-2 hr till it becomes clear solution. The solution is poured into test tubes and keeps it in water bath at 60^0C for 1hr or upto gel formation.

Take out test tubes from the water bath and break open the hydrogel and cut into slices of 1cm and then transfer the slices in the distilled water for removal of unreacted chemicals, stir initially and intermittently for 2 hr and keep it over night. Next day change the distilled water and stir it for 1hr. after that dry it in open atmosphere for 4-5 hr and then dry in vacuum oven at 60^0 cm till xerogel is formed [18].

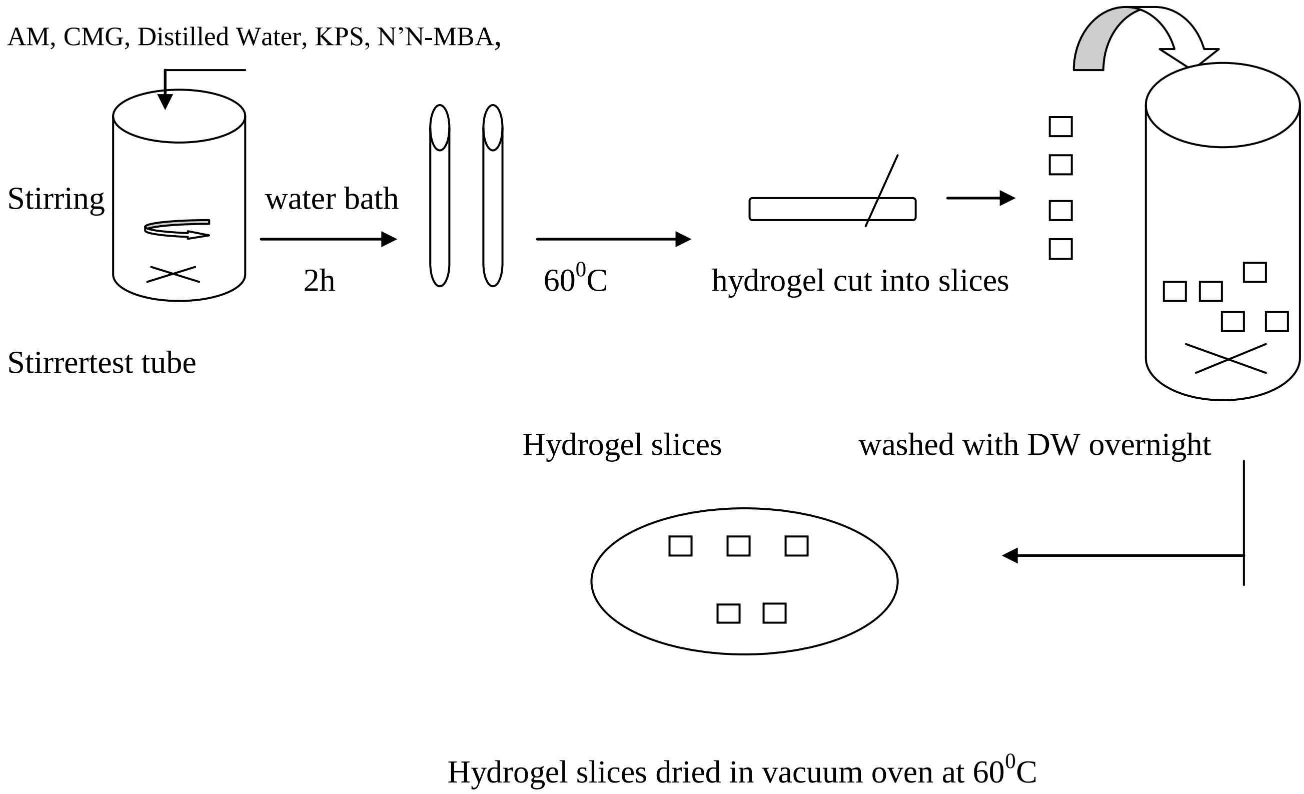


Fig.1 Flow diagram of hydrogel preparation [17]

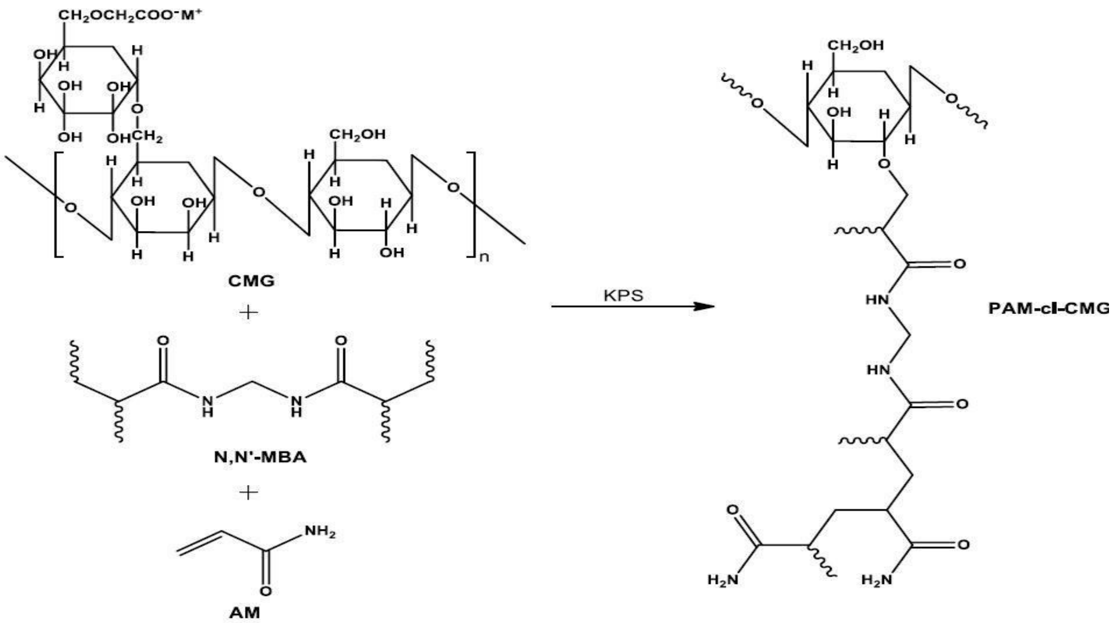


Fig-2 Synthesis of PAM/CMG [18]

Preparation of CMG/BPDM/Polyacrylamide hydrogel:

Take a beaker of 100ml and add 25 ml of distilled water in it. Weigh 2.5g of acrylamide and dissolved it in distilled in beaker, placed the beaker on magnetic stirrer. After that we add solution of acetone and Benziporphodimethene (BPDM). After that weigh 1% of potassium per sulfate by weight of acrylamide (Am) and dissolved it in beaker contain solution, potassium persulfate used as initiate. After that weigh 0.5% MBA by weight of Am, we know that MBA is not dissolved immediately therefore we dissolved it in distilled water separately and then mixed it in beaker. After that 10% of CMG is weigh and add it in solution slowly.

The mixture is stirred approx 1-2 hr for clear solution .When the solution mixed properly means it form a complete uniform dispersed solution ,the solution is pour into test tubes and keep it in water bath at 60^0C for 1hr or up to gel formation.

Take out test tubes from the water bath and break open the hydrogel and cut into slices of 1cm and then transfer the slices in the distilled water for removal of untreated chemicals, stir initially and intermittently for 2 hr and keep it over night. Next day change the distilled water and stir it for 1hr. after that dry it in open atmosphere for 4-5 hr and then dry in vacuum oven at 60^0 cm till xerogel is form [17-18].

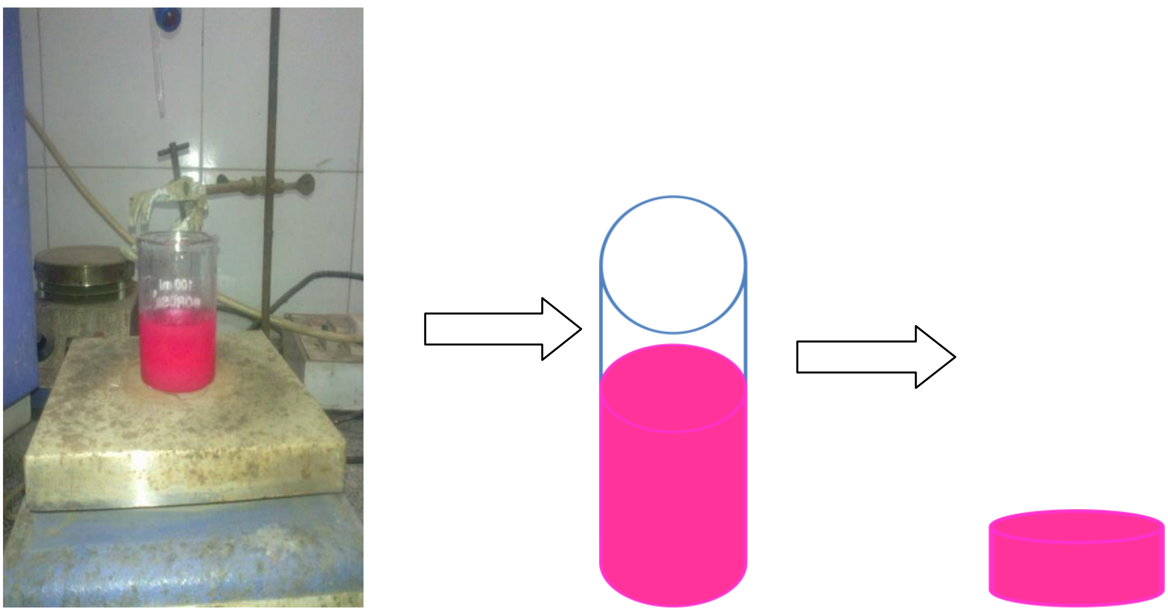


Fig. 3 Prepared hydrogel

S.No.	Name of sample	Acrylamide Am(g)	N,N′-methylene bisacrylamide (MBA)(g)	potassium persulfate (KPS)(g)	Carboxymethylated guar gum (CMG)	(BPDM) Compound (mg)
1	S1	2.5	0.0125	0.025	0.25	0.0
2	S2	2.5	0.0125	0.025	0.25	0. 5
3	S3	2.5	0.0125	0.025	0.25	1.0
4	S4	2.5	0.0125	0.025	0.25	1.5
5	S5	2.5	0.0125	0.025	0.25	2.0
6	S6	2.5	0.0125	0.025	0.25	2.5

Table 4: Sample that has been prepared.

CHARACTERIZATIONS TECHNIQUE:-

X-Ray Diffraction (XRD):

The basic objective of x-ray diffraction is to study the arrangement of polymer molecules in crystalline and amorphous regions forms. It is mainly used to determine the degree of crystallinity in polymers. X –ray techniques are based on the interaction between x-ray radiation and the specimen. Diffraction allows the study of structure of crystalline compound at atomic scale.

XRD categorized into two, depending on the angular regions of diffracted rays.

- Wide angle X-Ray diffraction
- Small angle X-Ray Diffraction

Wide angle diffraction is mostly used and it covers a broad range of bragg's angle 2ϴ from $2^0$to 60^0 and small angle diffraction are carried out from a few seconds of arc up to a degree.

Small angle x-ray scattering are used to study the structural and textural properties of two phase system (colloids,gel) at a larger scale. Diffraction pattern determine the chemical composition or phase composition of the film, the crystalline size, texture and the presence of stress. The graph represents the scattering intensity as a function of the 2ϴ angle. Every crystalline solid have a unique d-spacing pattern which is a figure print of solid.

$$\lambda = 2d \sin\Theta$$

Fourier Transform Infrared Spectroscopy (FTIR):

FTIR used to identify the chemical bonds in organic and inorganic molecules by producing an infrared absorption spectrum that is like a molecular "fingerprint". It is used to determine the components of unknown mixture. The type of sample used are solid, liquid and gasses.

It is a sample technique and is widely used for analysis and determination of polymer structure. The FTIR determine the chemical bonds in a molecule by producing an infrared absorption spectrum. The FTIR generates scan infrared spectral of samples that absorb infrared light. Metals do not absorb infrared light, but polymers that contain metals can be scanned with FTIR.

Spectra were taken on a Perkin Elmer paragon 500 FTIR spectrophotometer in a range of 400-4000 cm-1

Importance of FTIR

- It used to identify a unknown materials
- It can identify the amount of component in a mixture
- It can determine the consistency and quality of a sample

Depending on the element and the types of bond, molecular bonds vibrate at various frequencies. For any given bond there are many specific frequencies at which it can vibrate these frequencies vary from ground state to excited state .

For transition between two state the light energy required must be equal to the difference in the energy between two states means difference of energy of excited state and ground state.

Sampling Techniques Include;

- Transmission
- Solvent extraction
- Thin films
- KBr pellets

Sample preparation

Solid sample mixed with IR grade potassium bromide (KBr) in the ration of 1:100 to form a fine powder,then compressed into a thin pellet for analysis.

Atomic force microscopy (AFM):-

AFM is a high-resolution surface analytical; scanning probe microscopy type technique for studying structural and mechanical properties of hydrogels.AFM provides a three-dimensional surface sketch. The AFM contain a cantilever and at its end it has a sharp tip (probe) used to scan the specimen surface. The cantilever is made of silicon or silicon nitride and radius of curvature of tip is in nanometer. When the tip comes in contact of the sample surface, it generates a force between the sample and the tip lead to cantilever deflection according to Hooke's law. Forces that are measured in AFM depending on the situation include mechanical contact force, vander Waals forces, chemical bonding, capillary forces, magnetic forces, electrostatic forces etc [10].

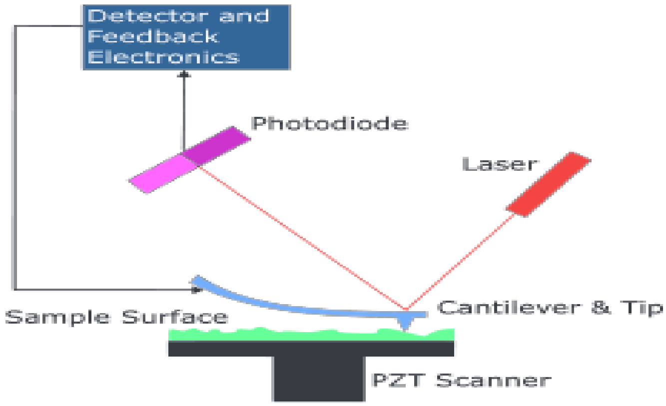


Fig 4: Block diagram of atomic force microscopy

Imaging modes are:

1. Contact mode
2. Non contact mode
3. Tapping mode

Scanning electron microscopy (SEM):

SEM is a versatile instrument used for analyzing the microstructure characteristics of a solid material. It use electron beams for creating magnified image of surface of a solid sample.

SEM used for Examination of surface morphology, porous material, multi component polymer etc.SEM can be used to provide information about the hydrogel surface topography, composition, and other properties such as electrical conductivity, porosity etc. Magnification in SEM can be controlled over a range of up to 6 orders of magnitude from about 10 to 500,000 times. It is widely used technique to capture the typical 'network' structure in hydrogels.

Thermogravimetric analysis (TGA):

It is a thermal analysis techniques used to measure change in physical and chemical properties in a sample as a function of increasing temperature or as a function of time at constant heating rate or constant temperature respectively. In TGA the weight loss cause because of decomposition, desorption, vaporization, sublimation, Break down reactions etc

The measured weight loss curve or TGA curve gives information about Changes in sample composition, Thermal stability and Kinetic parameters for chemical reactions in the sample.

Heating rate, sample size, gas flow rate and packing effect the TG curve [10].

Ultraviolet visible spectroscopy (UV-Vis):

UV spectroscopy is type of absorption spectroscopy in which light of ultra-violet region (200-400 nm.) is absorbed by the molecule. Absorption of the ultra-violet radiations results in the excitation of the electrons from the ground state to higher energy state. The energy of the ultra-violet radiation that are absorbed is equal to the energy difference between the ground state and higher energy states.

when a beam of monochromatic light is passed through a solution of an absorbing substance, the rate of decrease of intensity of radiation with thickness of the absorbing solution is proportional to the incident radiation as well as the concentration of the solution.

Swelling measurement:-

Swelling in distilled water:

Dried hydrogel pieces were used to determine the degree of swelling. The Swelling ratio (S_R) was determined by immersing the hydrogels sample in distilled water (50 ml) and was allowed to soak water at room temperature. After every 10min for 1hr then after every 30min for 4hr then after every 1hr upto saturation point is reached, they were removed from the water, blotted with filter paper to remove surface water and then weight the hydrogel.

The swelling ratio (S_r) was calculated using the equation below:

$$Sr = \frac{(Ws - Wd) * 100}{Wd}$$

Where,W_d and Ws are the weights of the samples in dry state and swollen in water respectively.

Effect of Temperature on swelling:

Dried hydrogel pieces were used to determine the degree of swelling. The Swelling ratio (S_R) was determined by immersing the hydrogels sample in distilled water at different temperature and was allowed to soak. After every 10min for 1hr then after every 30min for 4hr then after every 1hr, they were removed from the water, blotted with filter paper to remove surface water and then weight the hydrogel.

The swelling ratio (S_r) was calculated using the equation below:

$$Sr = \frac{(Ws - Wd) * 100}{Wd}$$

Where, W_d and Ws are the weights of the samples in dry state and swollen in distilled water at different temperature solution respectively.

Effect of P_H on swelling:

First form the solution of different P_H (2, 4, 6, 7, 10). Dried hydrogel pieces were used to determine the degree of swelling. The Swelling ratio (S_R) was determined by immersing the hydrogels sample in different P_H solution and was allowed to soak solution at room temperature. After every 10min for 1hr then after every 30min for 4hr then after every 1hr, they were removed from the water, blotted with filter paper to remove surface water and then weight the hydrogel.

The swelling ratio (S_r) was calculated using the equation below:

$$Sr = \frac{(Ws - Wd) * 100}{Wd}$$

Where,W_d and Ws are the weights of the samples in dry state and swollen in different P_H solution respectively.

HEAVY METAL ION DETECTION PROCESS:

The detection of heavy metal ions in aqueous solution was performed. The varied concentration of solutions of metal salt in distilled water was prepared.

Preparation of salt solution:

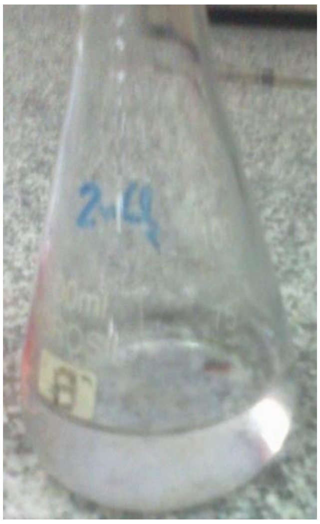
Weighed the required amount of metal salt, and then drop it in beaker or conical flask, after that measure 50ml distilled water and drop it in the beaker containing metal salt, mixed it properly. The metal salt solution was equally divided into two beakers or in conical flask.

Blank and compounded hydrogel was put in the metal salt solution separately. Both the solutions were kept in observation for 4hr. The detail analyses are given in the following Table.

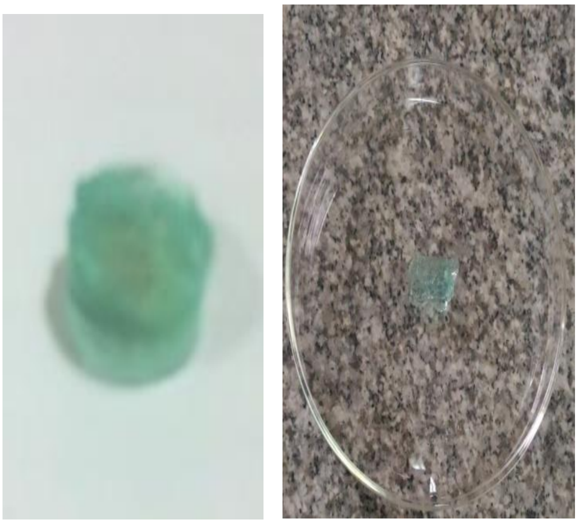
The following number of metal salt solution is formed:

Sl no.	Name of metal salt	Molar concentration (M)	Colour of solution	Effect on Blank hydrogel in salt solution	Effect on Compounded hydrogel in salt solution
1	Sodium chloride	0.05	colorless	No effect	No effect
2	Ferric Nitrate	0.05	yellow	No effect (colour of hydrogel change into yellow because of dyeing effect)	No effect (colour of hydrogel change into yellow because of dyeing effect)
3	Cobalt chloride Hexa hydrate	0.05	pink	No effect	No effect
4	Calcium chloride	0.05	colorless	No effect	No effect
5	Mercuric nitrate	0.05	Colorless	No effect	No effect
6	Cadmium nitrate	0.05	Colorless	No effect	No effect
7	Lead nitrate	0.05 M	Colorless	No effect	No effect

8	Zinc chloride	0.05 M	Colorless	No effect	Color change into **greenish blue** because of presence of zinc ions



$ZnCl_2$ solution with BPDM embedded hydrogel



hydrogel after Zn^{2+} absorption

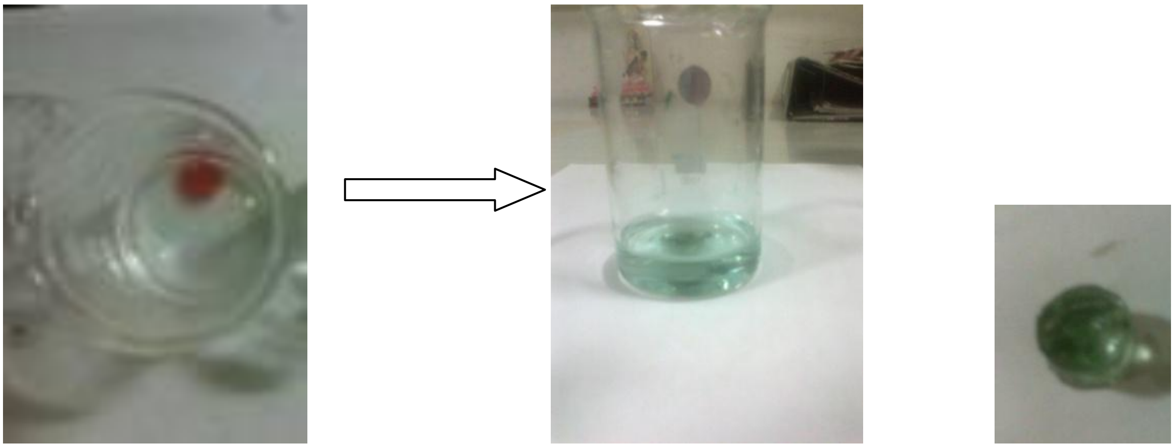
Fig 5: Hydrogel in $ZnCl_2$ solution in distilled water.

Interference test:

The effect of the interfering ions on the analysis of Zn^{2+} ions in aqueous solution was investigated in the presence of some common interfering metal salt solution such as Sodium chloride, calcium chloride, lead nitrate, mercuric nitrate. It was found that in 0.05M concentration of each metal salt in a mixture, it had no interference for Zn^{2+} ions detection because the solution formed is colorless solution. It does not shown any dying effect. But the solution of metal salt containing a salt solution having color like ferric nitrate it shows interference for the detection of Zn^{2+} ion because of dying effect. The hydrogel shows the color of the solution which contains metal salt as discussed above.

Effect of highly acid solution on compounded hydrogel:

When a highly acidic solution (pH = 0.78) containing the compounded hydrogel, after 17hr we saw that the color of hydrogel change into green and also the solution color turn green from colorless. This may be due to leaching of the same amount of compound into the water and acidified BPDM is water soluble.



Before After 17 hr hydrogel

Fig.6 Image of hydrogel in acidic solution (HCl)

Absorbance of leached out solution show a narrow peak at 407 nm and another peak at 603 nm. And Absorbance of the solution of BPDM in acetonitrile with 3 drops of dilute solution of HCl in 20ml distilled water. The spectrum shows the presence of leached out molecule followed by its oxidation.

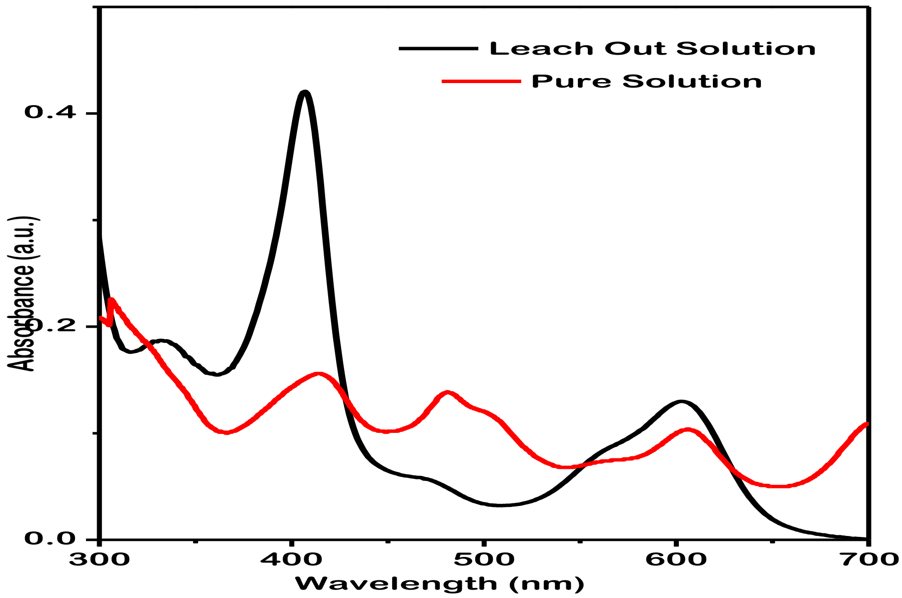
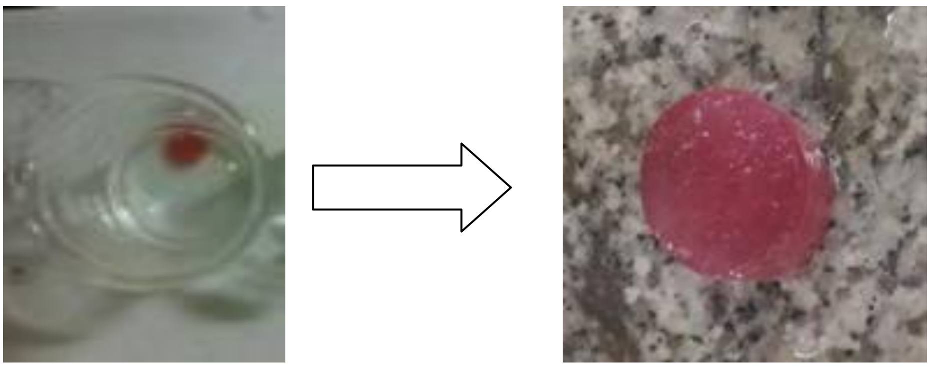


Fig 7: UV-Vis spectra of leach out and pure solution.

Effect of basic buffer solution on compounded hydrogel:

For identifying the effect of basic buffer solution on compounded hydrogel, first prepare a solution of basic buffer like ammonia buffer of pH=10. After that put the dried sample of compounded hydrogel in the buffer solution, after 8hr see the result. It does not show any effect like change of colour or leaching of compound in the solution from the hydrogel.



Before After

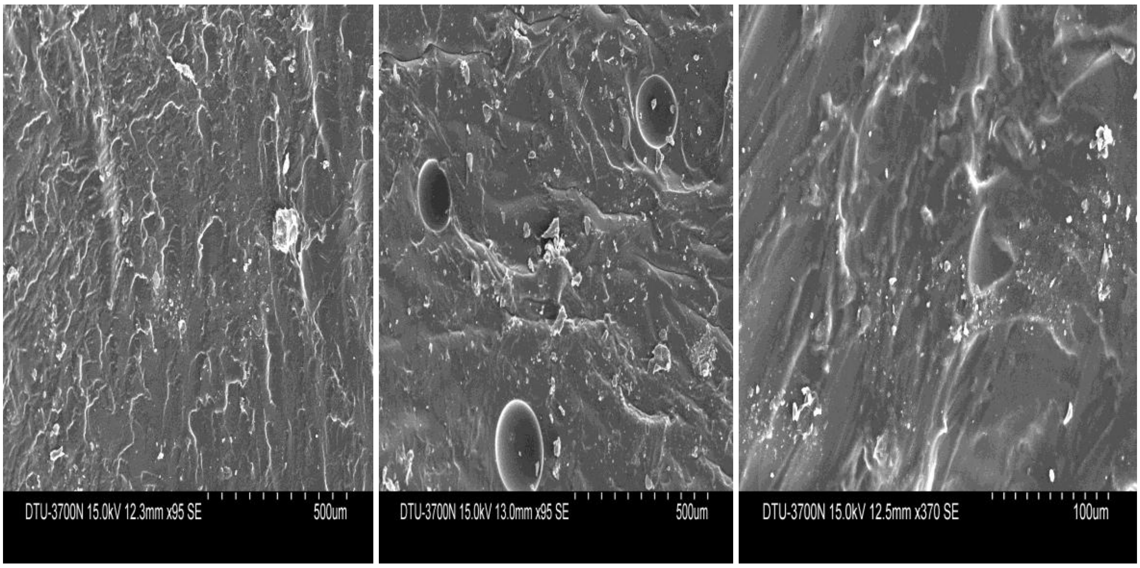
Fig.8 Image of hydrogel in basic solution.

Chapter-4

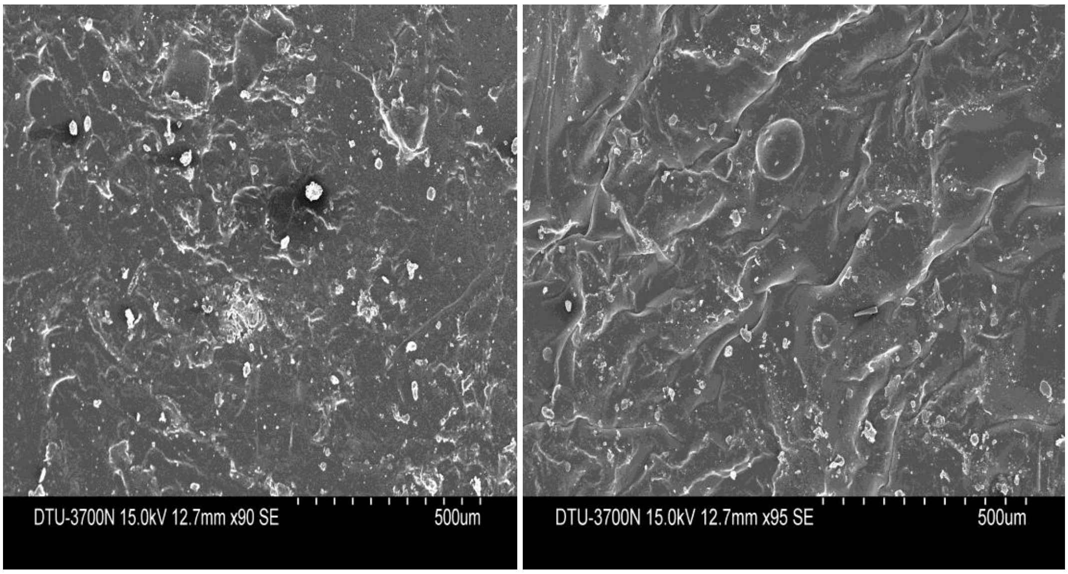
Result and discussion:

Scanning electron microscopy (SEM):

Surface morphology of BPDM embedded PAM/CMG and PAM/CMG hydrogels were investigated and compared using Scanning Electron Microscope (Hitachi S3700N Microscope). The images show that the hydrogel formed is a non-porous hydrogel. PAM/CMG hydrogel had an uneven and coarse surface with fibrillar structure and the imbedding of BPDM in the hydrogel doesn't show any effect on the surface morphology. Because of the non-porous nature of blank hydrogel, no change was observed when embedded with BPDM [18].



Sample 1 **sample 2** **sample 3**



Sample 4 **sample 5**

Fig.9. SEM image of different prepared sample

Fourier Transform Infrared Spectroscopy (FTIR):

The FTIR spectra of PAM/CMG hydrogel and BPDM embedded PAM/CMG hydrogel are given below. The stretching and deformation vibrations of BPDM embedded hydrogel (identical to the parent PAM/CMG hydrogel) were observed at 3438.9 cm-1 (O-H stretching), 2923.6 cm-1 ((C-H stretching), 1651.1 cm-1(amide-I, C-O stretching and amide-II, N-H stretching), 1455.2 cm-1 (N-H and C-N in-plane bending of amide) [11].

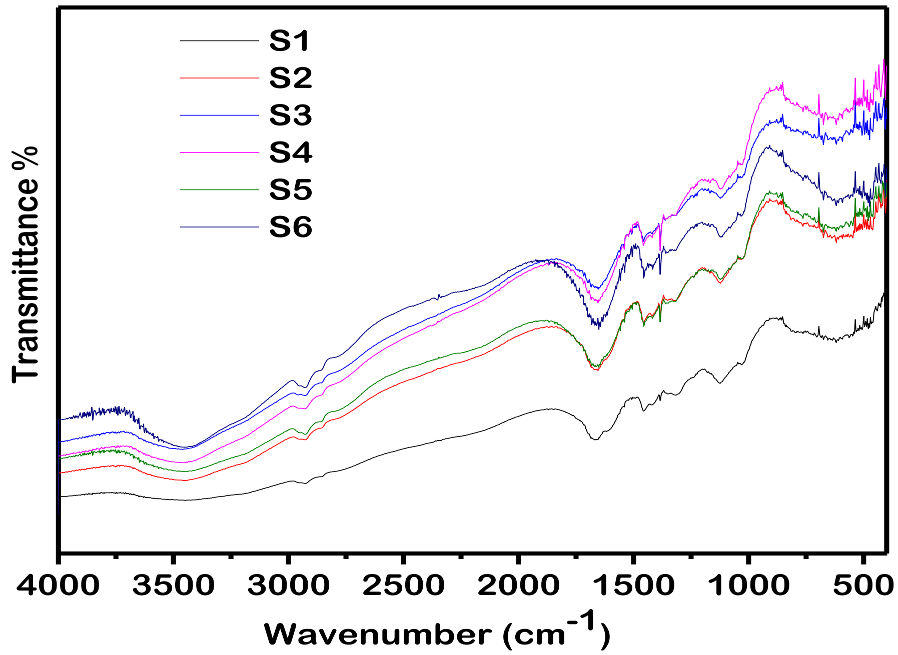


Fig.10. shows FTIR of comparison with blank and prepared sample

TGA (thermogravimetric analysis):

TGA represent the graph between the weight loss and the temperature. The temperature ranged from room temperature to 800 ^{0}C. TGA analysis of PAM/CMG and PAM/CMG/BPDM hydrogel are shown below in Figure. The data represent that the little amount of ash contain increase. In compounded hydrogel change in graph shown because of degradation/oxidation of BPDM at temperature around 70 ^{0}C.

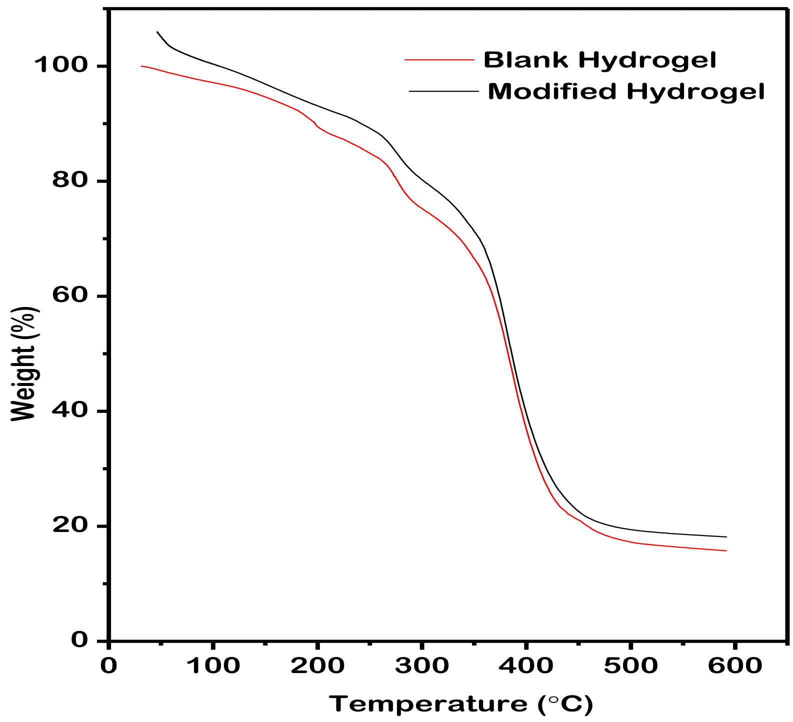
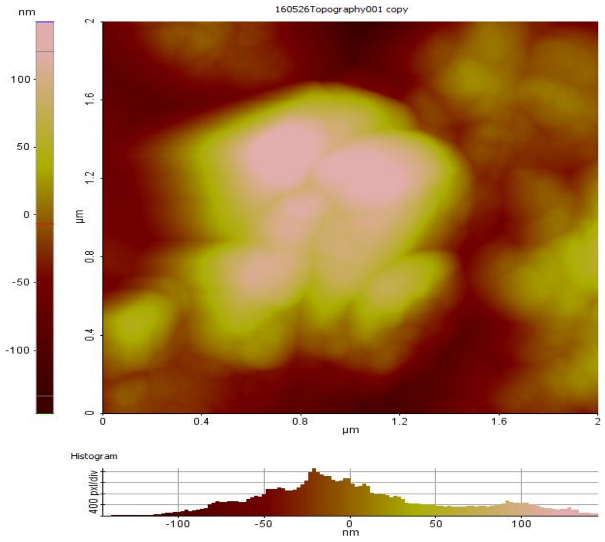


Fig. 11 shows the TGA reults of blank and BPMD modified hydrogel

AFM (atomic force microscopy):

The surface topography and irregularities of chemically treated samples can be identified from AFM. The light portion specify hydrophilic channels which help proton transfer and the darken portions symbolize the base matrix [26].



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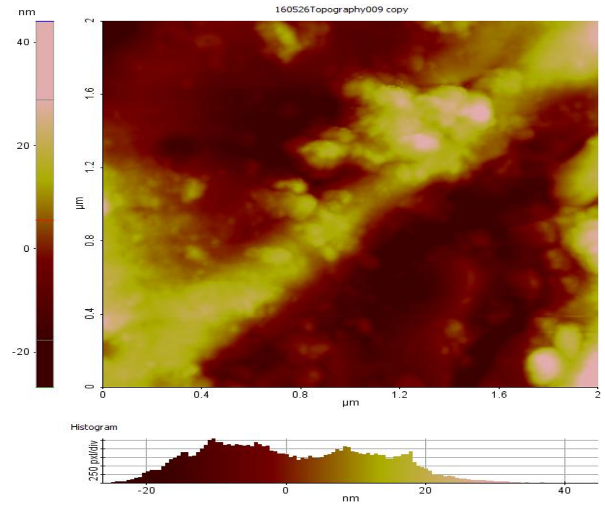
Amplitude	20.27 (nm)
Sel. Frequency	302.41E3 (Hz)
Drive	49.25 (%)

Blank hydrogel sample



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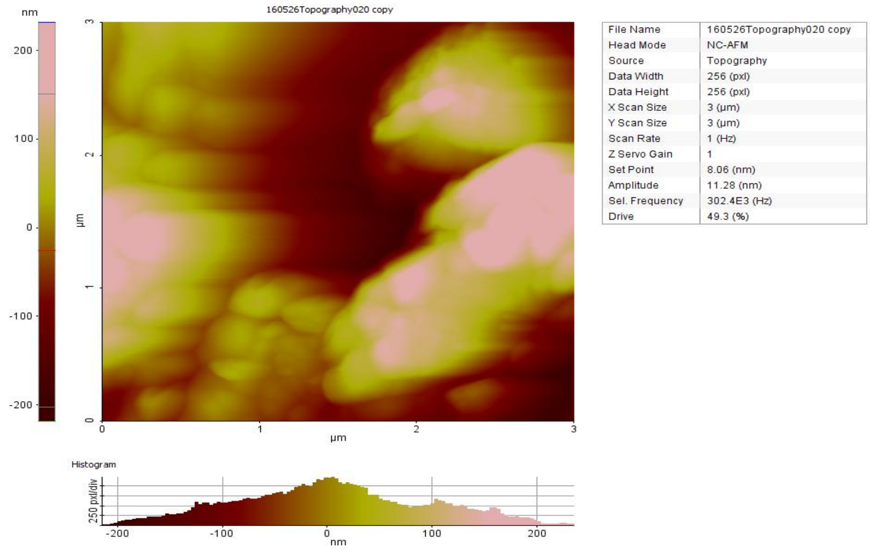
Drive	49.3 (%)

BPDM 1mghydrogel sample



BPDM 2.5mghydrogel sample

X-Ray Diffraction (XRD):

From 16 to 30 2ϴ degree values, a broad peak is observed in the blank samples which are retained in the corresponding 1 and 2.5 mg samples. However, an emergence of new small broad peak is observed from 39 to 44 degree values of 1 mg sample. Although the same broad peak flattens in the case of 2.5 mg. Moreover, an emergence of new small sharp peak is observed in the case of 2.5 mg at 26.83 degree. Additionally, a shift towards sharper peak is observed over the range of 16 to 30 2ϴ degree values in the case of 2.5 mg sample. All these observations suggest a shift in crystalline planes of the samples in comparison to blank [22].

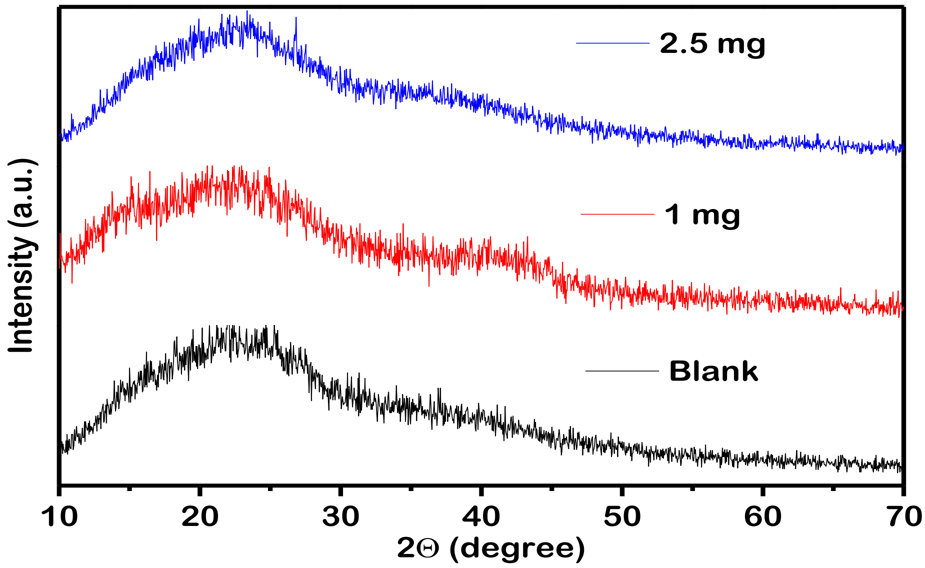


Fig 15: XRD graph of blank and modified hydrogel.

Swelling effect:

Effect of pH on swelling:

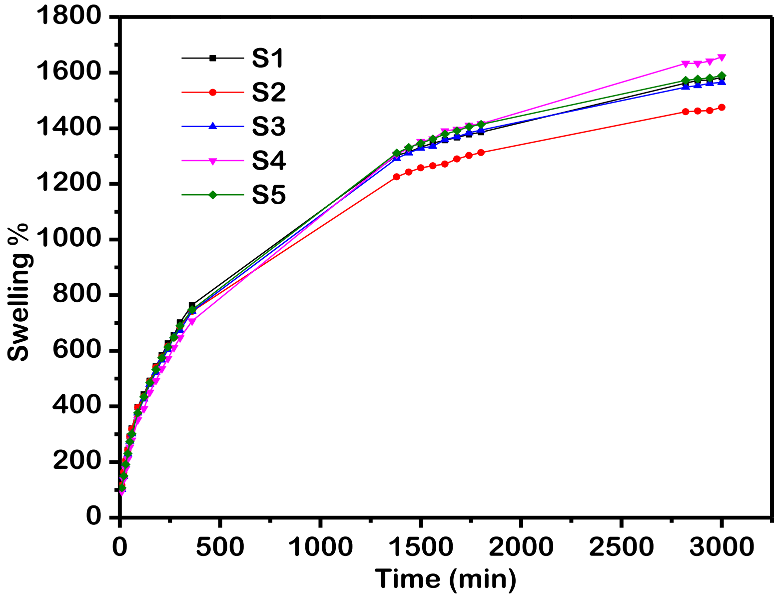
The swelling ratio (S_r) was calculated using the equation below:

$$Sr = \frac{(Ws - Wd) * 100}{Wd}$$

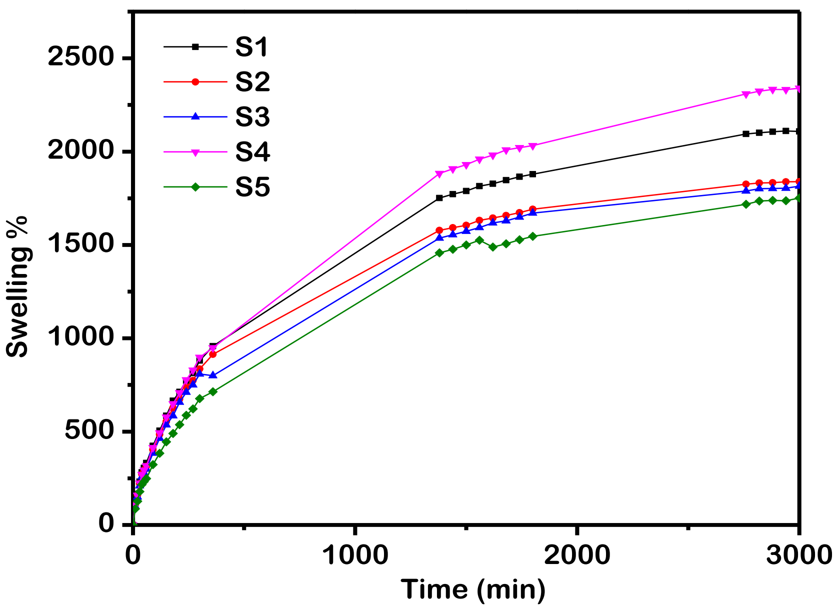
Where, W_d and Ws are the weights of the samples in dry state and swollen in water, respectively.

The pH effect on swelling of blank hydrogel and compounded hydrogel are represented below. It shows that at pH 7, it shows maximum absorbance or swelling then acidic or basic pH and least swelling at pH 2 [23]. The addition of BPMD in hydrogel doesn't effect on swelling.

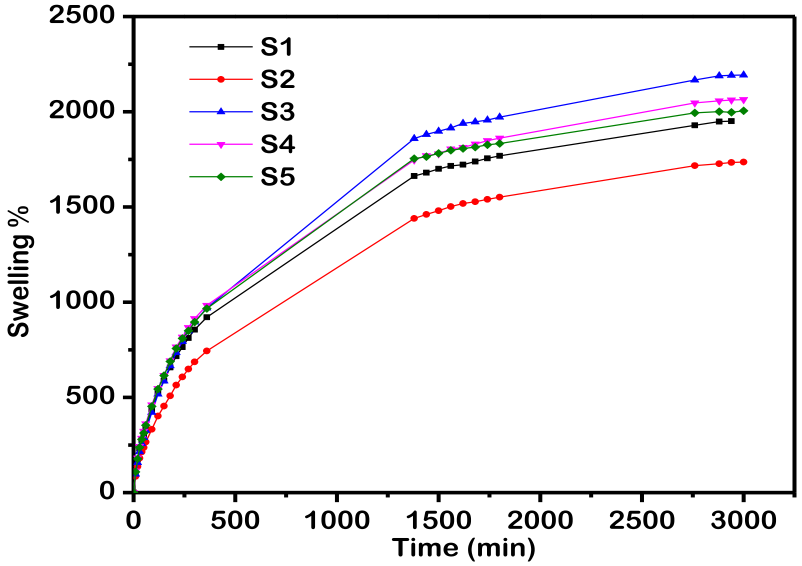
Swelling at pH 2



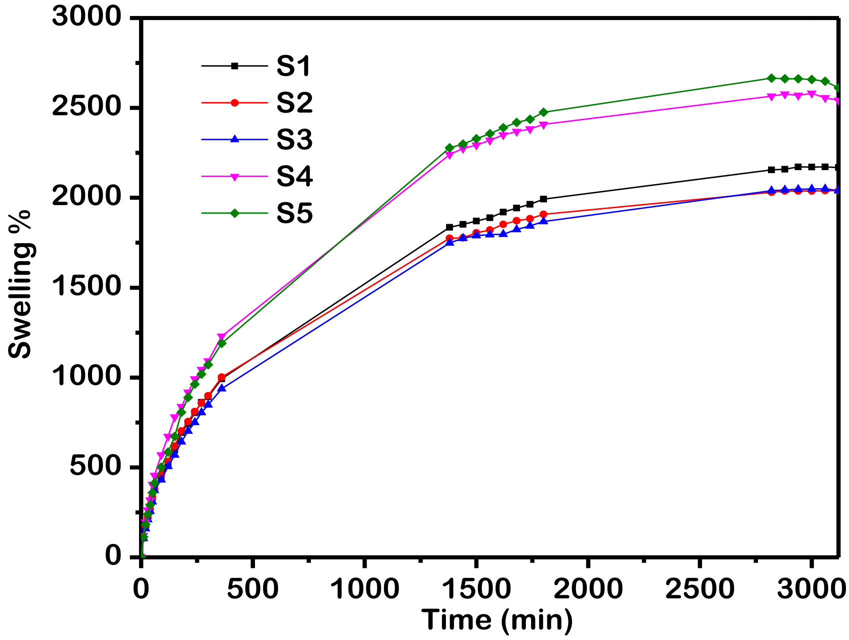
Swelling at pH 4



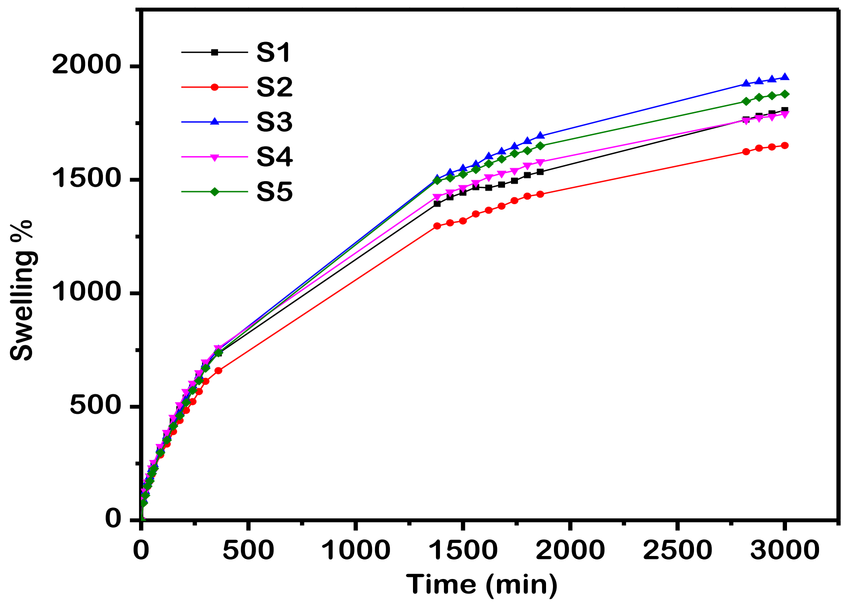
Swelling at pH 6:



Swelling at pH 7



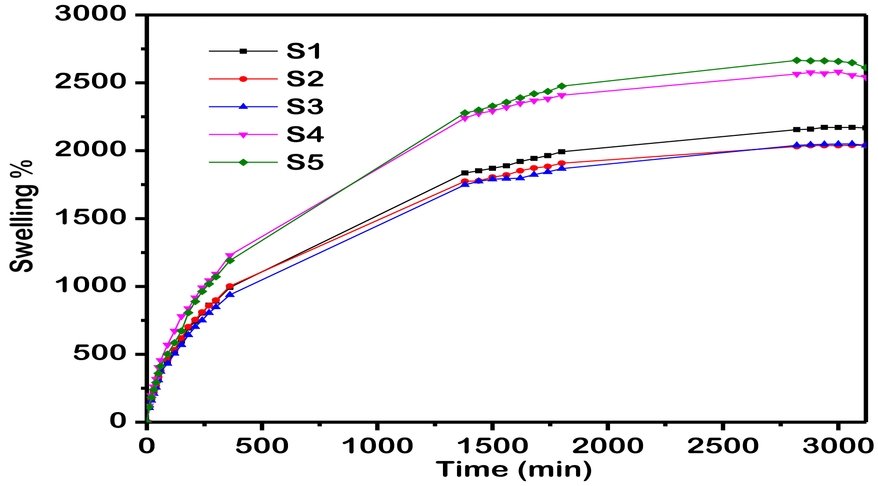
Swelling at pH 10:



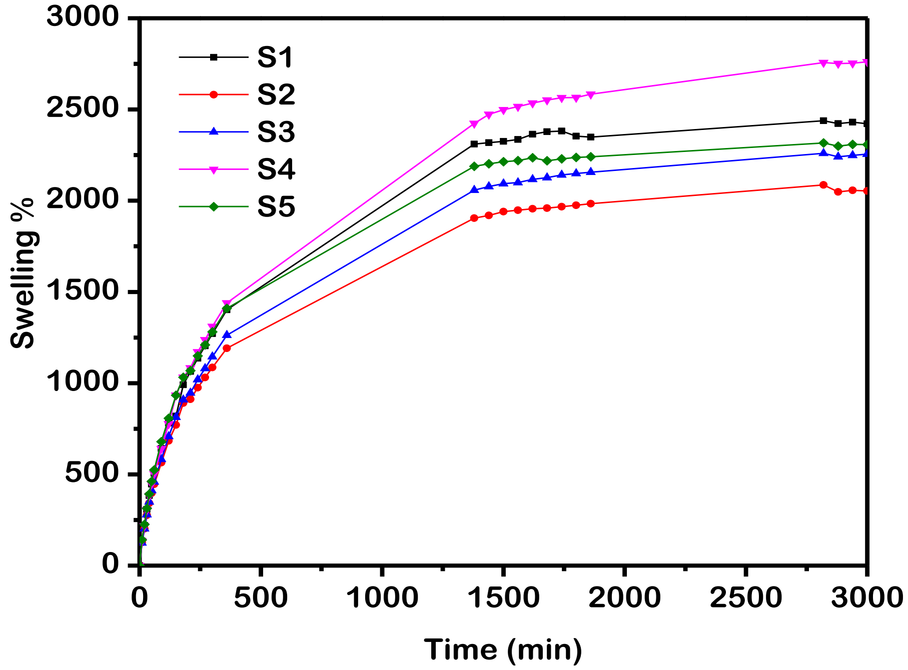
Temperature effect on swelling

The swelling behavior of polymer network is influenced by the temperature of the swelling medium. The increase in the temperature of the swelling medium is usually accompanied by the increase in the swelling rate of the hydrogels due to increased kinetic energy of solvent molecules. It shows that as we increase the temperature the swelling is increase. The presence of BPDM doesn't effect on swelling.

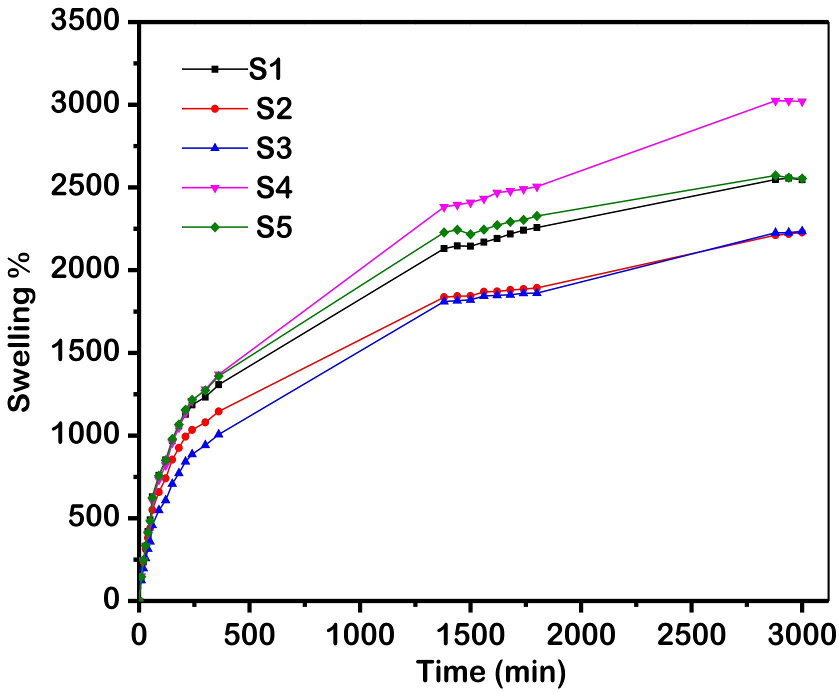
Swelling at temp 35^0C



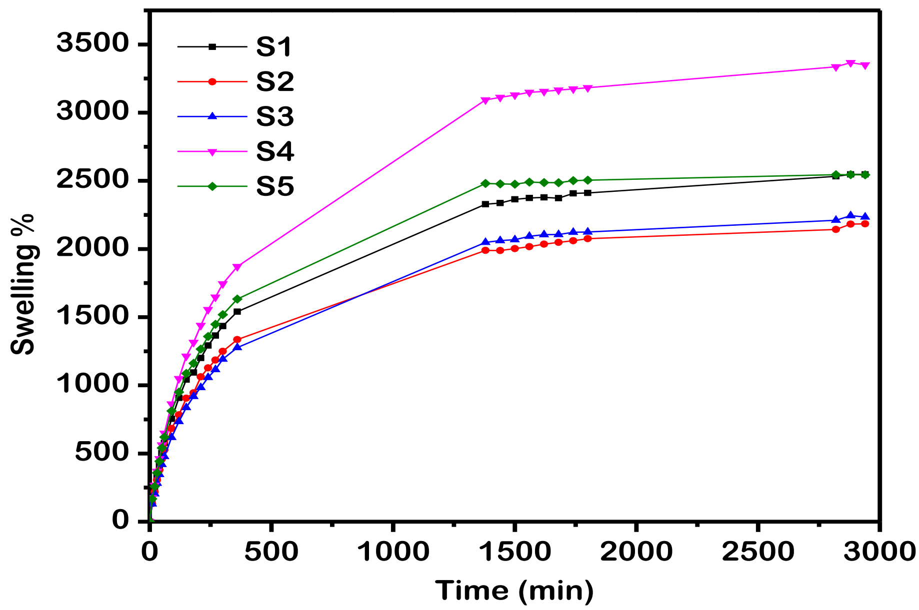
Swelling at temp 40^0C



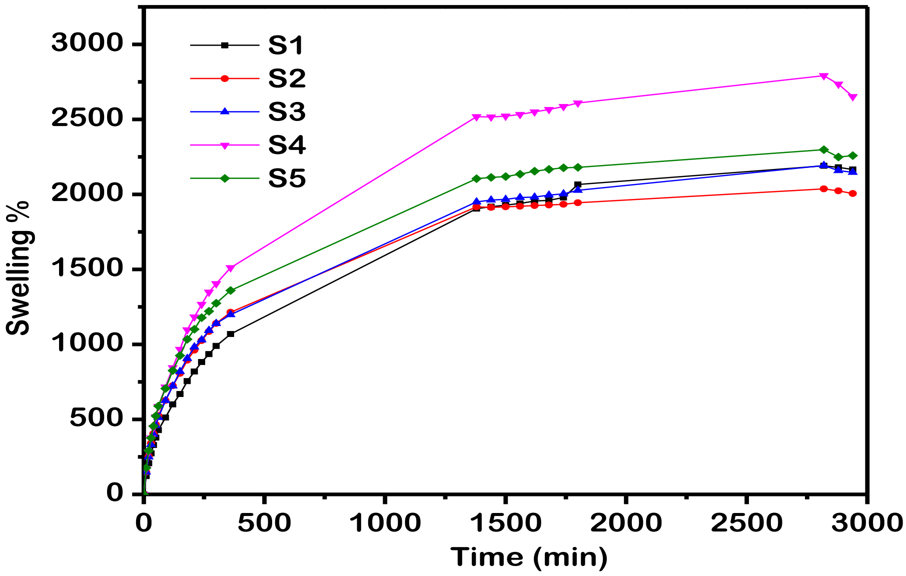
Swelling at 45^{0}C



Swelling at 50^{0}C



Swelling at 55^0C



Chapter-5

CONCLUSION:-

Biodegradable hydrogel based on poly acrylamide/carboxymethylguargum were synthesized by in situ polymerization using potassium persulphate as an initiator and N'N-methylbisacrylamide as cross-linking agent and a fluorescent indicator BPDM for detection of Zn^{2+} ion in aqueous solution. The synthesized PAM/CMG hydrogel were characterized by a variety of techniques like TGA, FTIR, SEM, AFM, XRD and UV Vis spectroscopy.

Swelling results shows that as the increase in temperature the rate of swelling increase. The swelling rate was observed maximum at pH 7. Also, the included compound BPDM get degraded above temperature 55^0C.

The modified hydrogel successfully detect the presence of Zn^{2+} ion in the aqueous solution. The detection limit of the sensor is 0.001M solution of $ZnCl_2$.

Chapter-6

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