PREPARATION AND CHARACTERIZATION OF INTERPENETRATING HYDROGEL FOR COLON DRUG DELIVERY

A Thesis submitted in partial fulfilment of the requirements for the degree

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By

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CERTIFICATE

This is to certify that the thesis entitled, "***PREPARATION AND CHARACTERIZATION OF INTERPENETRATING HYDROGEL FOR COLON DRUG DELIEVERY***" submitted by **Ms. Shusmita Kumari** in partial fulfillment of the requirements for the award of the degree of **Master of Technology** degree in **Biomedical Engineering** at National Institute of Technology, Rourkela is an authentic work carried out by him under my supervision and guidance.

To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other university/institute for the award of any degree.

Place : Rourkela

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ABSTRACT

This thesis work is based on preparation and characterization of Interpenetrating (IPN) hydrogels of poly (HEMA) with polysaccharides (xanthan gum or isabgol). IPN hydrogels were prepared by cross-linking the HEMA in the presence of varying concentration of xanthan gum or isabgol. The concentrations of xanthan gum used for this purpose were 0.1% (w/v), 0.3% (w/v),0.5% (w/v) and 0.7% (w/v) whereas the concentrations of isabgol selected were 1% (w/v), 2% (w/v), 3% (w/v) and 4% (w/v). The HEMA and the polysaccharides either xanthan gum or isabgol were mixed in the proportion of 30:70 (w/w) before being croslinked using APS and TEMED. The IPN hydrogels were characterized by swelling tests at Ph 1.2, pH 7 and Ph 9.Physical properties were assessed by XRD and FTIR. Suitability of its application as colon drug delivery system (CDDS) was assessed by incorporating curcumin for *in-vitro* drug release kinetics assay in the simulated condition of gastrointestinal system. The IPN hydrogels were found to swell maximal at pH 7 and the rate of drug release was found to be highest at pH 6.8 indicating its potential application as CDDS.

Keywords: Interpenetrating hydrogels, xanthan gum, isabgol, HEMA, colon drug delivery.

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Abbreviations

Abb.	Description
Conc.	Concentration
%	Percentage
μl	microlitre
w/v	Weight/volume
Hr	hour
Min	minute
Nm	nano meter
Gm	gram
w/w	weight/weight
FTIR	Fourier Transform Infrared Spectroscopy
XRD	X-Ray Diffraction Spectroscopy
FESEM	Field Emission-Scanning Electron Microscope
^{o}C	Degree Centigrade
pH	Hydrogen ion concentration
NaCl	Sodium Chloride
Rpm	Rotation per minute
APS	Ammonium Persulphate
OD	Optical Density

IPN	Interpenetrating network
SGF	Simulated Gastrointestinal Fluid.
SIF	Simulated Intestinal Fluid.

CHAPTER 1 : INTRODUCTION

1.1: Introduction

1.1.1 Hydrogels

Hydrogels are three dimensional (3D) polymeric networks that can retain large amount of water inside it. They are super retaining polymeric materials which have huge parts in social insurance particularly for wound medication/ assurance. This may be because of their hydrophilicity, biocompatibility and biodegradability. Hydrogels has numerous wonderful properties, for example, quick agony control impact, simple substitution, transparency, obstruction against microbes, great grip, oxygen penetrability and retention. From the human services purposes of view, hydrogel dressings have turned into an exceptionally fascinating field of examination for the improvement of an easy to use medicinal gadget for humanity. Various examination studies demonstrate that a damp wound environment is best for wounds to mend [1]. The hydrogels holding both hydrophilic and hydrophobic sections on the macromolecular chains present amphiphilic (affiliated) fascinating properties, controlled by their hydrophilic/hydrophobic offset. Hydrogels could be artificially steady or reversible (physical gels) stabilized by atomic snares, as well as auxiliary strengths including ionic, H-holding or hydrophobic connections, these hydrogels being non-homogeneous. Examples of reversible hydrogels are "ionotropic" hydrogels structured by the collaboration between a polyelectrolyte and an oppositely charged multivalent particle, and the polyelectrolyte edifices (complex coacervates) framed by the communication between two oppositely charged polyelectrolytes. Physical gels might be deteriorated by progressions in nature's domain conditions, for example, ionic quality, ph, and temperature. Physical hydrogels have various biomedical provisions in pill conveyance, wound dressing, tissue designing etc. Covalently cross-joined systems structure lasting or concoction gels ""Smart"" hydrogels can essentially change their volume/shape because of little modifications of specific parameters of nature's turf. Responsive hydrogels have various

provisions, the majority of them being cantered around organic and remedial requests, and sensing requisitions. On the other hand, single-system hydrogels have powerless mechanical properties and moderate reaction at swelling.Different procedures from material science, microscale designing and microfluidics have been utilized to synthesise biomimetic hydrogels[2].

1.1.2 IPN

These IPN hydrogels are composites of cross joined polymers, at least one of them being orchestrated as well as cross-connected inside the quick vicinity of the other without any substance securities between them, which cannot be differentiated unless the concoction securities are broken [3].There are various hydrogels focused around the polysaccharides (chitosan, alginate, starch, and different polysaccharides like xanthan gum, isabgol, accasia gum.) and protein based IPN hydrogels. These interpenetrating polymer network hydrogels (IPN) have picked up a great deal of consideration in the late years, primarily in view of their biomedical requisitions. They have provisions in medication conveyance and detachment forms, this is focused around the way of systems they have.

1.1.3 Poly (HEMA)

There has been a significant improvement in the field of 2-hydroxyethyl methacrylate (HEMA). HEMA is the monomer of the polymer poly (2-hydroxyethylmethacrylate). This polymer has the exceptional property to get swelled because of the particle's hydrophilic pendant gathering when subjected to water. It is equipped for retaining water from 10 to 600% in respect to the dry weight. As a result of its extraordinary property, it was one of the first materials to be utilized as a part of contact lenses. Poly (2-hydroxyethyl methacrylate) orpoly (HEMA) is a standout amongst the most widely mulled over hydrogels utilized as a part of biomedical provisions. A

thermoset that is not enzymatic ally corrupted or hydrolysed by acidic solution. Various studies have been directed to alter poly (HEMA) with the point of enhancing its mechanical properties. Its electro responsive properties supposed to inspire better physiological responses[4].

1.1.4 Xanthan Gum

Xanthan gum is a polysaccharide emitted by the bacterium *Xanthomonascampestris*. There have been a considerable measure of work done on Xanthan gum; it has great thickening properties because of which it is used as a rheology modifier. Xanthan gum is regularly utilized as a nourishment thickening operator (in greens dressings, for instance) and a stabilizer (in nonessential items, for instance, to keep fixings from differentiating)[5].. It can also be used as an emulsifier because it has both hydrophilic and hydrophobic ends which bind with oil-water interface.

1.1.5 Isabgol

Isabgol are the psyllium seed husk otherwise called isabgula. They are the shares of the seed of plant Plantagoovota. They are the local of India and Pakistan [6], [7]. They are hygroscopic means they can pull in and hold water atoms from the nature's turf, which permits them to grow and get adhesive. It is a thick, gluey substance generated by almost all plants and a few microorganisms. It is a polar glycoproteins and an exopolysaccharides. Exopolysaccharides are high sub-atomic weight polymers that are made out of sugar deposits and are emitted by microorganism into the nature's domain. Isabgol are unpalatable and are a wellspring of solvent dietary fibre. They are utilized to mitigate stoppage, peevish inside syndrome and diarrhea[8] . Isabgol husk adhesive is a reasonable dry gelling executor. It can expand in volume by engrossing water up to 40 times its weight. It comprises of 85% of water solvent fibre and acts by hydration in the gut. It is made up of polysaccharides; it is prominently utilized as a mass

diuretic. Its cytoprotective movement has been demonstrated by distinctive biotic investigations. It is essentially utilized for its emollient impact. It has been demonstrated that it additionally demonstrates against diabetic impact. It might be likewise utilized as a part of colorectal tumour, ulcerative colitis, haemorrhoids, and hypercholesterolemia and so on. Isabgol is a common hotspot for both solvent and insoluble fibre which are vital for fitting assimilation. The solvent fibre show in isabgol turns into a marginally mucous like substance when blended with water that we drink with isabgol [9]. This substance cleans the gastrointestinal tract of undigested nourishment, fats sugar and common poisons which are structured from the sustenance we consume. Along these lines isabgol helps in detoxification and makes us livelier. It likewise helps in weight reduction if normally utilized once as a part of a month. Since, it follows up on the digestive organs, so it expands our metabolic rate. Isabgol helps in diabetic control, insulin stacked with polymer micro-particles made out of crosslinked poly(methacrylic corrosive) and poly(ethylene glycol) are multi-utilitarian bearers, and have indicated high insulin fuse proficiency, a quick insulin discharge in the digestive tract, chemical-hindering impacts[10], [11]. Polysaccharides based hydrogels upgrade the intestinal assimilation of insulin and expand the relative pharmacological bioavailability of insulin[6,8]. Gooey types of dietary fiber have been demonstrated to enhance blood glucose control by trapping ingested sugars inside the thick gel framed after assimilation. Therefore, sugars are retained into the circulatory system all the more gradually, restricting the ascent in blood glucose seen after a feast . So isabgol could be utilized as a great natural solution[12].

They have the effectiveness for improving both the thickness and the strength of emulsion rely on upon the polymeric fixation and structure [13]. There have been a considerable measure of examination work done on hydrogels, in these years the exploration interest has been moved to jolts responsive frameworks, which can change their physical properties because of outside

triggers, for example, temperature, ph, ionic quality, light or different outer fields. Specifically, a ton of techniques exists to join micrometer to centimetre scale of synthetic inclinations and complex biomaterials consolidating such gradients[14].

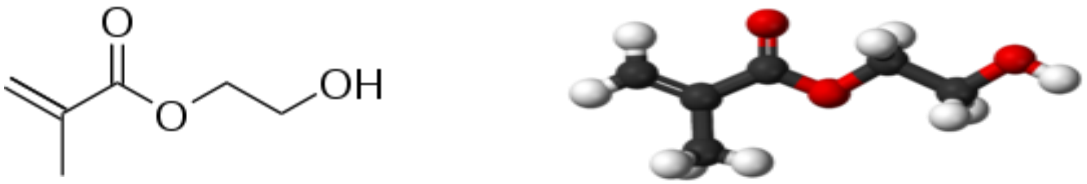
This thesis consists of preparation and characterization of two interpenetrating hydrogels (IPNs) used for various biomedical applications.In both the IPNs HEMA is used as base material whereas Xanthan gum and isabgol was added in varying concentrations before being cross-linked with APS and TEMED.

1.2.Objectives:

- To prepare the IPNs of xanthan gum with HEMA
- To prepare the IPNs of isabgol with HEMA
- To characterize these IPNs by using different characterization technique
- To study the *in vitro* drug kinetics mimicking the condition of gastrointestinal system

CHAPTER 2 : LITERATURE REVIEW

Hydroxyethylmethacrylate or HEMA is the monomer that is utilized to make the polymer poly (hydroxyethylmethacrylate). The polymer is hydrophobic; nonetheless, when the polymer is subjected to water it will swell because of the atom's hydrophilic pendant gathering. Contingent upon the physical and substance structure of the polymer, it is equipped for retaining from 10 to 600% water in respect to the dry weight. In light of this property, it was one of the first materials to be effectively utilized within the assembling of adaptable contact lenses.



(a) (b)

Fig 2.1 a) Molecular Structure of Hydroxyethylmethacrylate (HEMA) b) It's ball – and – stick model *(Source : Wikipedia)*

Zhang et. al. (2013) describes the antifouling qualities of the polysulfone half and half ultrafiltration layer ready with Tio_2-g-HEMA. The polymeric material polysulfone (PSF) is generally utilized within ultrafiltration layers as a result of its great mechanical properties, solid synthetic dependable qualities and wide pH operation range. On the other hand, the utilization of polysulfone in medication of water is limited because of its hydrophobicity property which prompts serious layer fouling and decay of porousness. Consequently, it is important to adjust the PSF membrane to enhance its hostile to-fouling execution. Hybrid layers framed by mixing TiO_2 nanoparticles and natural materials are appealing for making new materials with improved properties, for example, high permselectivity, great hydrophilicity and fabulous fouling safety in an extensive variety of applications. In request to improve the hydrophilicity of films and to defeat the agglomeration of nanoparticles in films and spillage of nanoparticles from

nanocomposite layers in the water medication process, polymer chains of HEMA (2-hydroxyethylmethacrylate) were developed from TiO_2 by the iota exchange radical polymerization (ATRP) handle in methanol. By including distinctive proportion of TiO_2 (T layers) and TiO_2-g-HEMA (HT membranes) particles by means of stage reversal incited by the inundation precipitation method, the mixture polysulfone (PSF) films were then ready. The adjustments of nanoparticles were found to give clear changes in decreasing the particles agglomeration and fouling in PSF membranes. Different strategies, for example, SEM, TEM, FTIR, EDS, contact point goniometry and filtration analyses of water, BSA and EPS were connected to describe and investigate the execution of particles and layers. Contrasted and unmodified Tio_2 particles, adjusted Tio_2 particles held improved hydrophilicity, better similarity and dispersibility inorganic dissolvable and lattice polymer. Essentially, the HT membranesexhibitedsuperiorwaterfluxandanti-pollution result in separation with the pure PSF membrane and T membranes[15].

Yang et al. (2012)made a copolymeric bioadhesive skeleton with the possibility to be used as a tissue paste centred around biopolymer dextran. Copolymeric hydrogels including a urethane dextran (Dex-U) and 2-hydroxyethyl methacrylate (HEMA) were primed and crosslinked under the ultraviolet (UV) light. In this study, the photo polymerization system was checked by constant infrared spectroscopy (RTIR). The hold quality was surveyed by lap-shear-test. The surface pressure, thickness of the results and the cytotoxicity tests were analyzed. Appeared differently in relation to Dex-U schema, the extension of HEMA vitally upgraded the properties of Dex-H structure especially the hold quality and the nontoxicity. The copolymeric tissue pastes displayed ensuring grasp quality and nontoxicity.

Ansteinsson et al. (2011)describes theDNA-damage, cell-cycle arrest and apoptosis induced in BEAS-2B cells by2-hydroxyethyl methacrylate (HEMA).The methacrylate monomer 2-hydroxyethyl methacrylate (HEMA) is customarily used as a piece of gum-based dental medicinal materials. These materials are cured in situ and HEMA and distinctive monomers have been perceived in including air all through dental surgery. In vitro studies have exhibited a dangerous capability of methacrylates, and concerns have been raised regarding possible wellbeing effects on account of internal breath. In this study, the systems of HEMA-affected threat in the human lung epithelial cell line BEAS-2b. Depletion of cell glutathione (GSH) and an extended level of sensitive oxygen species (ROS) were seen after 2 h of presentation; however the levels were restored to control levels after 12 h. After 24 h, limited cell duplication and apoptotic cell destruction were found. [16].

Aghaie et al. (2009) studies the adsorption features of Cu^{2+} and Pb^{2+} ions onto Poly(HEMA) andP(MMA-HEMA) surfaces from aqueous single solution.The adsorption merits of Cu^{2+} and Pb^{2+} particles onto poly2-hydroxyethyl methacrylate (PHEMA) and copolymer 2-hydroxyethyl methacrylate with monomer methyl methacrylate P(mma-HEMA) adsorbent surfaces from watery single result were investigated concerning the progressions in the pH of result, adsorbent piece (changes in the weight rate of MMA copolymerized with HEMA monomer), contact time and the temperature in the different fluid results. [17].

Shantanu Dhara et. al (2013) describes the synthesis and characterization of a hydrogel based on dextrin grafted with poly(2-hydroxyethyl methacrylate) by using N,N-methylene bis acrylamide (MBA) as cross linker, into a polymeric network in the existence of potassium persulphate (KPS) initiator for colon specific delivery of ornidazole. Hydrogels [Dxt-g-p(HEMA)] prepared with different concentration have been synthesized by altering the reaction

parameters and the best one optimized. The hydrogel was then characterized using FTIR spectra, 13C NMR spectra, elemental analysis, XRD study, SEM analysis, TGA analysis, swelling study and cell viability study. The equilibrium swelling ratio of the hydrogels has been examined in different media and found that it is maximum at pH 7.4. The study of cell viability indicates that the hydrogel is non-cytotoxic in nature. The results of drug delivery show that Dxt-g-p(HEMA) delivers ornidazole effectively in the colonic region in a controlled way.The drug release mechanism and kinetics of ornidazole from different hydrogels have been investigated using linear and nonlinear mathematical analysis, which verify that ornidazole release from hydrogel follows first order kinetics and the mechanism of non-Fickian diffusion [18].

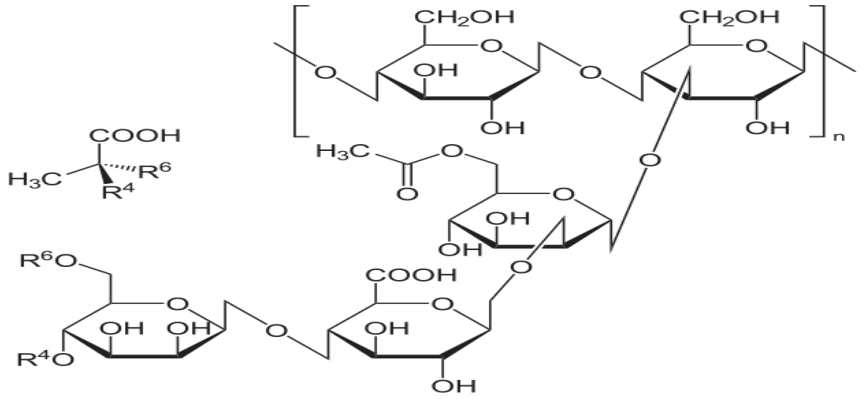


Fig 2.2 Molecular Structure of Xanthan gum ($C_{35}H_{49}O_{29(monomer)}$)
(Source : Wikipedia)

According to **Hironori Izawa et. al. (2013),** xanthan gum (Xan) hydrogel indicating superb mechanical properties. Mineralization of hydroxyapatite (Hap) upon the Xan hydrogel might give a remarkable biomaterial pertinent to bone tissue building. The mineralization of Hap upon the Xan hydrogel by method for an exchange splashing methodology. Hap was step by step developed upon the Xan-lattice surface with expanding number of splashing cycles because of the ionic connections between calcium cations and carboxyl gatherings. Interestingly, the mineralization incited a microstructure change in the gel-framework from a layered structure to a

permeable structure. The mechanical properties of the ensuing Hap–Xan composite hydrogels were further examined by a ductile test[19].

Zare et. al (2011) describes the effects of molasses concentration, agitation rate and media temperature on the yield of fermentation in xanthan gum production process were iexamined . Xanthan gum was formed in batch fermentation by Xanthomonas campestris PTCC 1473 from molasses. At 31 °C, 600 rpm and media with 32 g/l of total sugar, maximum production of xanthan gum (17.2 g/l) was attained. For the clarity of the xanthan FTIR spectrum was accquired. The recognized spectrum was compared with the viable product. In batch culture, numerous kinetic models for the biochemical reactions were extensively studied. The growth kinetic parameters were investigated by unstructured model and derived from the correlated equations. Based on Malthus and Logistic rate equations, the maximum specific growth rate, μmax, and initial cell dry weight, X_0, were defined. Luedeking-Piret and Modified Luedeking-Piret models were applied for the product development and substrate utilization rates. In batch experiments, the kinetic parameters for the growth associated (m, a) and non-growth associated (n, b) parameters were examined [20].

Singh et. al (2008) developed the hydrogels meant for the drug delivery, we have prepared psyllium-N vinylpyrrolidone (NVP) based hydrogels by radiation induced crosslinking. Polymers were characterized with SEMs, FTIR and swelling studies. Swelling of the hydrogels was studied as a function of monomer concentration, total radiation dose, temperature, pH and [NaCl] of the swelling medium. The swelling kinetics of the hydrogels and release dynamics of anticancer model drug (5-fluorouracil) from the hydrogels have been carried out for the evaluation of swelling and drug release mechanism. It has been observed that diffusion exponent ‘n’ have 0.8, 0.9, 0.8 and gel characteristics constant ‘k’ have 9.22, 10.3, 2.06, 10.3, 11.72, 10.3 values for the release of drug from the drug loaded hydrogels in distilled water, pH 2.2 buffer

and pH 7.4 buffer, respectively. The present study shows that the release of drug from the hydrogels occurred through Non-Fickian diffusion mechanism [21].

VaniaBlasques Bueno et. al. (2012) depicts thesynthesis and swelling conduct of xanthan-based hydrogels. Xanthan chains were crosslinked by esterification reaction at 165°C in presence of citric acid. Hydrogels swelling degree delayed at high ph values, as a result of electrostatic repulsion and ester linkages break. Harmony swelling degree was influenced by salts (NaCl), dependent upon gel creation and kind of salt. Effects could be illustrated by cooperation between particles and chain of polymer[22].

JiminGuo. et. al. (2014)showed the periodate oxidation of xanthan gum and its crosslinking effect on gelatin – based edible films.Oxidized xanthan gum with distinctive aldehyde substance is effectively ready by periodate oxidization and utilized as a crosslinking executor for gelatin consumable movies.The optical properties studies indicate that all films are extremely transparent and have fantastic hindrance properties against UV light. Presenting aldehyde gatherings can enhance the UV boundary properties, coming about because of the expanded Cdouble bond. With the increase in oxidation of xanthan gum, the improvement of water obstruction properties, mechanical properties and warm solidness of gelatin–oxidized xanthan gum films were observed. [23].

CHAPTER 3 : MATERIALS & METHODS

3.1 Materials

The chemicals used were as follows:

Table no. 1 : Chemicals used with their specification

Name of chemical	Chemical formula	Name of the company	Catlog no.
Xanthan gum	$C_{35}H_{49}O_{29}$ (monomer)	Himedia	GRM7618-100G
Isabgol or Psyllium Husk	-	Baidyanath	-
HEMA / 2-Hydroxyethyl methacrylate	$(C_6H_{10}O_3)$	Himedia	RM4710-500G
APS/Ammonium Persulphate	$(NH_4)_2S_2O_8$	Rankem	A0560-500G
TEMED / N,N,N,N-TeramethylEthylenediamine	$(C_6H_{16}N_7)$	SRL	202788-100ml

3.2 Methods

3.2.1 Preparation of IPNs of xanthan gum.

1.5ml IPN hydrogels of xanthan gum were prepared with HEMA. The different concentrations of xanthan gum were taken in amount of 0.1% w/v, 0.3% w/v, 0.5% w/v and 0.7% w/v.1ml of xanthan gum solution mixed with the 0.45 ml of HEMA. After that, 25µl amount of APS solution was mixed in it and then, the same amount (25µl) of TEMED solution was mixed for crosslinking purpose and after the formation of hydrogels they were kept in vacuum drying for 2 days.

3.2.2 Preparation of IPN of Isabgol.

1.5ml IPN hydrogels of isabgol were prepared with HEMA.The different concentrations of xanthan gum were taken in amount of 1% w/v, 2% w/v, 3% w/v and 4% w/v.1ml of isabgol solution mixed with the 0.45 ml of HEMA. Secondly, 25µl amount of APS solution was mixed

in it and finally, the same amount (25µl) of TEMED solution was mixed for crosslinking purpose and after the formation of hydrogels they were kept in vacuum drying for 2 days.

Different tests and characterization technquies used for classifying the properties of these interpenetrating hydrogels based on xanthan gum and isabgol using HEMA are as follows :

1. Swelling study
2. Moisture absorption
3. Haemocompatibility
4. X- Ray Diffraction (XRD)
5. Fourier Transform Infrared Spectroscopy (FTIR)
6. Field Emission Scanning Electron Microscopy (FESEM)
7. Drug Release Kinetics Test

3.3 Swelling study.

The swelling study of xanthan gum and isabgol hydrogel was carried out in three main pH parameters:

a) In pH 1.2 buffer solution of HCl.
b) In pH 7 solution (double distilled water)
c) In pH 9 buffer solution of Na_2HPO_4.

All these swelling studies were carried out for 1 hr, 2hr, 3hr, 6hr, 12 hr, 24 hr and 48 hr in body temperature of 37° C. The hydrogels of each concentration of xanthan gum and isabgol were made in triplets.

3.3.1 Swelling study in pH 1.2 buffer solution of hydrochloric acid (HCl) :

Firstly, 400ml of distilled water was taken in a beaker, added 1 gm of sodium chloride (NaCl) in it and then solution was stirred. And finally, hydrochloric acid (HCl) was added in the buffer

solution to make it a pH solution of 1.2 which is acidic in nature. Four different concentrations of xanthan gum were taken in duplicates. The four different concentration of 0.1% w/v, 0.3% w/v, 0.5% w/v, 0.7% w/v of xanthan gum and 1% w/v, 2%, 3%w/v, 4%w/v of isabgol were taken. The Hydrogels of each concentration were formed in triplets. The initial weight of the hydrogels were taken and after drying it for 2 days in vacuum at 37^oC their weight were measured for swelling study, then they were kept in the pH solution for 1hr, 2hr, 3hr, 6hr, 12hr, 24hr, 48hr subsequently and their weights were measured. The mean and standard deviation of each samples were taken out and plots was made between % of swelling mean versus time in hours.

3.3.2 Swelling study in pH 7 solution

This was done by keeping the samples of xanthan gum and isabgol hydrogel of different concentration in double distilled water for the respective hours. The hydrogels of each concentration of xanthan gum and isabgol were formed in triplets and their weights were measured was taken and again the plot was made between % of swelling mean versus time in hours

3.3.3 Swelling study in pH 9 buffer solution of disodium phosphate (Na_2HPO_4)

The pH 9 buffer solution is very much similar to pH value of bile juice found in human body, which helps in digestion and its basic in nature. The hydrogels of each sample of xanthan gum and isabgol were formed in triplets. Swelling study of different hydrogels of different concentration was performed in pH 9 buffer solution of disodium phosphate (Na_2HPO_4) .The mean and standard deviation of each samples were taken out and plots was made between % of swelling mean versus time in hours**.**

3.4 Moisture absorption :

This study was done in the presence of KCl. First weights of dried samples of each concentration of hydrogels of xanthan gum and isabgol was measured. Then these samples were put in a dissipater for 3 days, and their weights were measured after 24 hrs and 48 hrs and based on their moisture absorption graph was plotted between % of Moisture absorption and time.

3.5 Hemocompatability :

This test was done by taking goat blood. It was then diluted with NaCl in the ratio 1:1. Goat blood was taken in 20 ml, mixed with 20 ml NaCl to form diluted blood. The PBS (Phosphorous Buffer Solution) was prepared with NaCl = 0.8g, KCl = 0.02g, Na_2HPO_4 = 0.144g, KH_2PO_4 = 0.024g. This PBS is having a pH value of 7.4. Four different concentrations each of xanthan gum and isabgol were taken in 9 ml normal saline, mixed with 0.5ml diluted goat blood and 0.5ml leachants was added in a centrifuge tube and along with them two +ve control and two –ve control were also taken both for xanthan gum and isabgol separately. All these samples in the centrifuge tube were then shaken in an incubator with 600 rpm speed. The + ve control was made with 0.1(N) HCl = 0.5 ml and –ve control was prepared of 0.1 (N) NaCl =0.5 ml. Then their Optical Density Reading was taken in a wavelength of 545 nm using an instrument called Double Beam Spectrophotometer.

Then the % Hemolysis was calculated using the formula

$$\% \text{ Hemolysis} = \frac{T_s - N_s}{P_c - N_v} * 100$$

Where, T_s ---- Test Sample

N_s --- Negative Sample

P_C--- Positive Control

N_V ----: Negative Control

3.6 X- Ray Diffraction:

The hydrogels of different concentration of xanthan gum and isabgol were analyzed using X-ray diffractometer (PW3040, XRD-PANalytical, Philips, Holland).Cu – Kα radiation with wavelength 0.154 nm was used as a source. The instrument was operated at 30 KV and 20 mA.Scanning of the samples was done at 5° - 50° 2θ with a rate of 3°2θ /min. The analysis was performed at the room temperature.

3.7 Fourier Transform Infrared Spectroscopy :

The prepared hydrogels were examined for spectroscopic analysis using FTIR spectroscopy ATR mode (Shimadzu/IR prestige 21). These samples were analyzed keeping air as the reference. Reading of air was initially taken by the machine for background subtraction and then the samples were placed in machine to record FTIR readings, thus subtracting the peaks obtained by air. Scanning was done in the range of 4000 cm^{-1} to 500 $cm^{-1}$with a resolution of 4 cm^{-1}. The maximum absorption in HEMA is found at 1735 cm^{-1} and 1153cm^{-1} wavenumber of the Ester group .

3.8 Field Effect Electron Microscope (FESEM):

Here one sample of xanthan gum hydrogel of xanthan gum 0.7% conc and one sample of isabgol hydrogel of isabgol 4% conc. was taken to find the FESEM images. The instrument used for FESEM was NOVASEM 450 (imported from Czech Republic). The FESEM images were taken in high vacuum at 30kV. The samples were coated with gold sputtered coating so that the outer surface becomes conductive.

3.9 Drug Release Kinetics:

When a drug loaded capsule enters the body, it passes through three important pH values. First it has to pass through stomach where most of the carbohydrates, proteins, fats and other nutrients are digested having a acidic pH of 1.2 because of HCl then it enters duodenum which has a pH of 7.4 and then the colon has a pH value of 6.8. So I tried to mimic this situation by preparing IPN hydrogels of xanthan gum and isabgol loaded with the drug curcumin. The prepared hydrogels were then dissolved in ph 1.2 solutions for 2 hrs, then in pH 7.4 for 3 hrs and in pH 6.8 for 11 hours as per the protocol. Every hour 200 µl of the drug mixed pH solution was taken out and thus the aliquots for the OD reading were collected. Simultaneously the same amount of the fresh solution of respective pH was added. The OD readings of the collected sample were taken at 420 nm.

Simulated gastric fluid (without pepsin) solution of pH 1.2 was prepared. This fluid was prepared using HCl and NaCl. Phosphate buffer solution pH 7.4 was prepared to mimic the duodenum environment of the body. The composition of this PBS solution was NaCl, KCl, Na_2HPO_4, KH_2 PO_4. Now gastrointestinal fluid of pH value 6.8 was prepared to mimic the colonic environment. This fluid was prepared by dissolving monobasic potassium phosphate in distilled water and then 0.2N sodium hydroxide was added in it. First the wet samples of hydrogels of xanthan gum and isabgol were prepared including their controls which only contained HEMA by using curcumin as the drug and APS and TEMED as cross linkers and as initiators. Each sample was prepared of volume 1.5 ml. Four different concentrations each of xanthan gum and isabgol were prepared respectively. During the preparation of hydrogels, 1.0 ml of xanthan or isabgol polymer solution was taken, mixed with 0.45 ml of HEMA , then 1.5 mg of curcumin was added and vertexed properly. Then 25 µl of APS and 25 µl of TEMED solutions

were added to the polymer solution to form gel. After the wet samples were prepared, they were kept in respective pH solutions as per mentioned above. The OD readings at 421nm were taken using the Double Beam Spectrophotometer. Now % drug release was calculated from the OD readings collected and graph of curcumin absorption in mg/ml verses time in hours was plotted.

CHAPTER 4 : RESULTS AND DISCUSSIONS

4.1 Gelation Time:

The gelation time for xanthan gum hydrogel formation was found to be 7 to 8 min and the time for isabgol hydrogel formation was 6 to 7 mins.

4.2 Swelling study

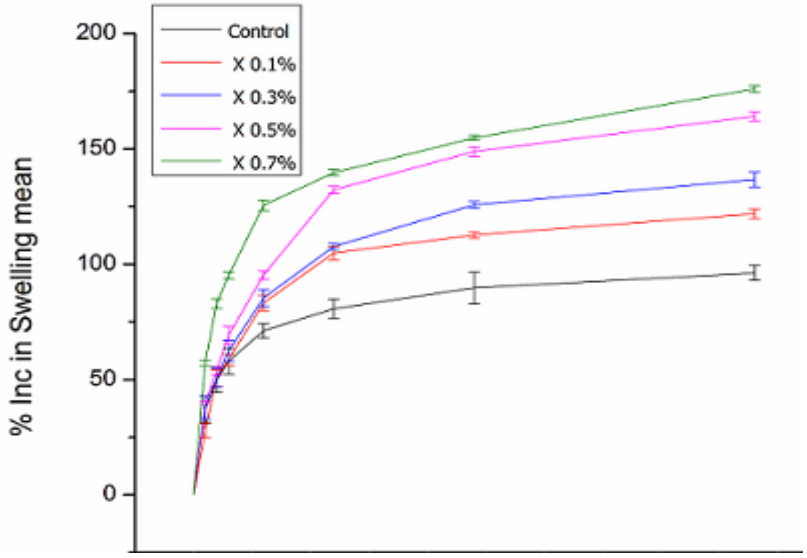
4.2.1 Xanthan gum hydrogel (at 37^{o}C)

$$\% \text{ Increase in Swelling } = \frac{F_w - I_w}{I_w} \times 100$$

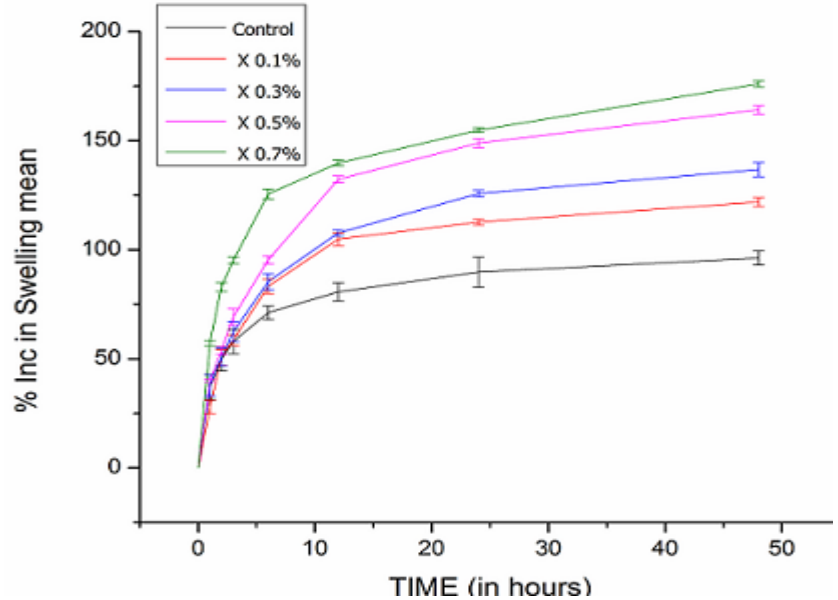
F_w : Final Weight of the xanthan gum hydrogel sample

I_w : Initial Weight of the xanthan gum hydrogel sample

a)



b)



c)

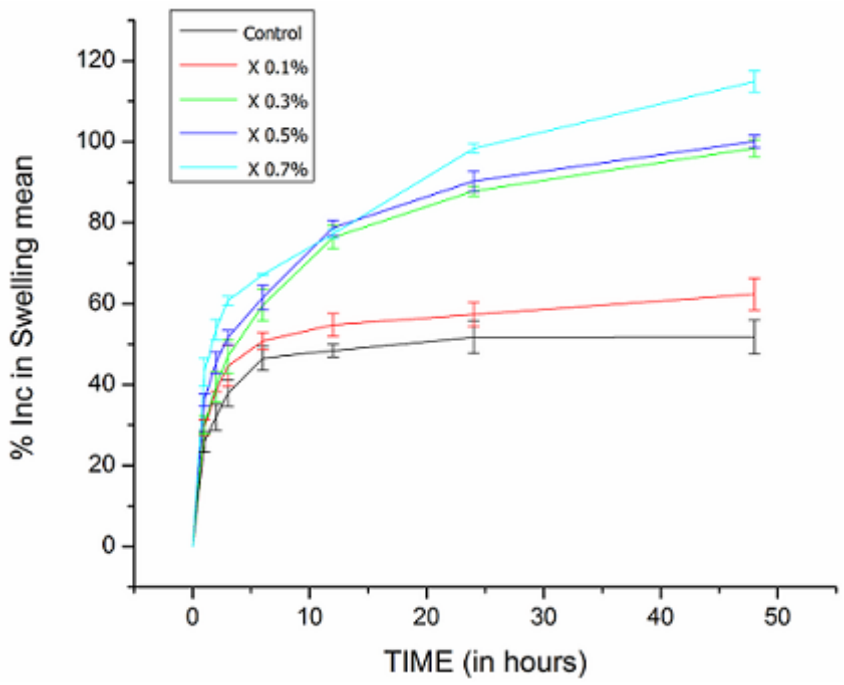


Figure 4.1 : Swelling study plot of Xanthan gum hydrogel at a) pH 1.2, b) pH 7, c) pH 9 at different concentration

Figure 4.1 a) reveals the following results of the swelling study og xanthan gum hydrogel at pH 1.2 :

- The maximum swelling is shown by xanthan gum hydrogel of 0.1% xanthan gum w/v with a % increase in swelling mean of 139%.
- The second highest swelling is shown by xanthan gum hydrogel of 0.7%% xanthan gum w/v conc. with a % increase in swelling mean of 131%.
- The third highest swelling is shown by xanthan gum hydrogel of 0.5%% xanthan gum w/v conc.with a % increase in swelling mean of 128%
- The least swelling took place in xanthan gum hydrogel of 0.3% w/v conc. with a % increase in swelling mean of 111% .
- The control is showing a % swelling mean of 80%

- Swelling effect : X- 0.1%> X 0.7% > X 0.5% > X 0.3%> control

It was found from the experiment that the xanthan gum interpenetrating polymer network hydrogel swells more at acidic conditions than the poly(HEMA) gel, that means this IPN gel can be used for delivering drug at more acidic conditions or than gum hydrogel is more pH sensitive than HEMA hydrogel.

Similary figure 4.1 b) and c) shows that :

- At pH 7
    - ❖ The maximum swelling is shown by xanthan gum hydrogel of 0.7% xanthan gum w/v conc.with a % increase in swelling mean of 166%.
    - ❖ The second highest swelling effect is shown by xanthan gum hydrogel of 0.5% xanthan gum w/v conc.with a % increase in swelling mean of 151%.
    - ❖ The third highest swelling effect is shown by xanthan gum hydrogel of 0.3% xanthan gum w/v conc. with a % increase in swelling mean of 121%.
    - ❖ The least swelling took place in xanthan gum hydrogel of 0.1% xanthan gum w/v conc.with a % increase in swelling mean of 111%.
    - ❖ The control is showing a % swelling mean of 60%
    - ❖ Swelling effect : X0. 7% > X 0.5% > X 0.3% > X 0.1 % > control.

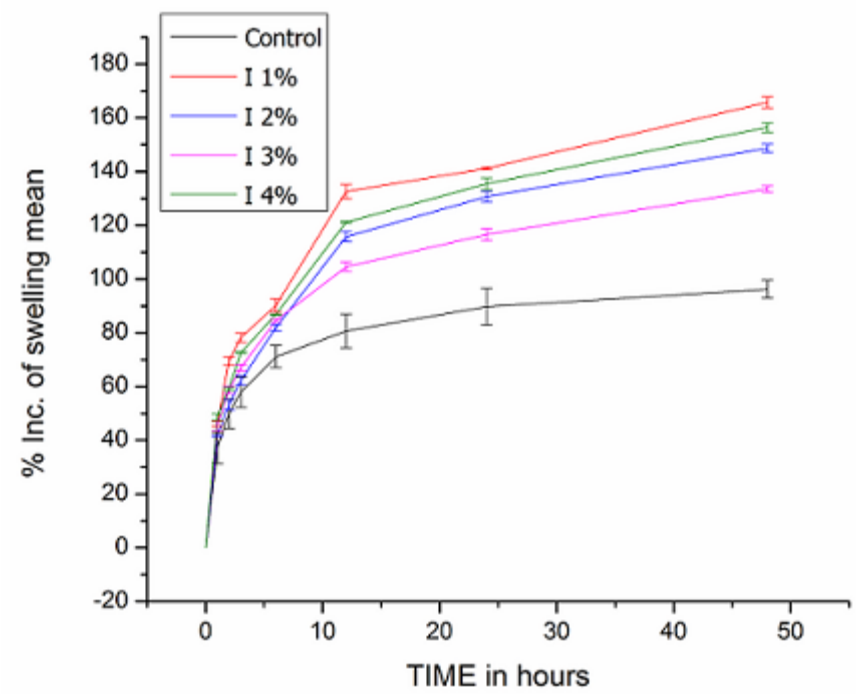
Also it is interesting to note that the swelling of gels at neutral pH is more than the acidic pH but that is not so in case of poly(HEMA) gel which swells in acidic pH more than the neutral pH. This maybe because of in pH 7 xanthan gum polymeric chains are more charged which lead to dispersion within the chains and hence more swelling takes place.

- And at pH 9
    - ❖ The maximum swelling is shown by xanthan gum hydrogel of 0.7% xanthan gum w/v conc.with a % increase in swelling mean of 115%.
    - ❖ The second highest swelling effect is shown by isabgol hydrogel of 0.5% xanthan gum w/v conc.with a % increase in swelling mean of 95%.
    - ❖ The third highest swelling effect is shown by isabgol hydrogel of 0.3% xanthan gum w/v conc. with a % increase in swelling mean of 91%.
    - ❖ The least swelling effect is shown by xanthan gum hydrogel of 1% xanthan gum w/v conc. With a % increase in swelling mean of 51%.
    - ❖ The control is showing a % swelling mean of 51%
    - ❖ Swelling effect : X 0.7% > X 0. 5% > X 0.3% > X 0.1 % > control.

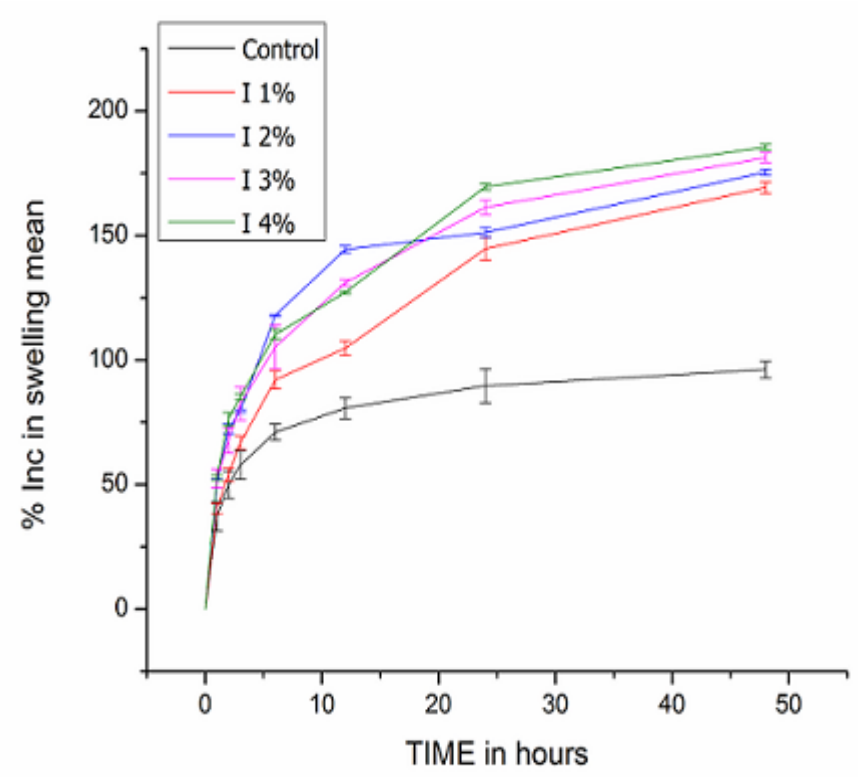
On comparing between the swelling at acidic, neutral and basic pH, it can be observed that maximum swelling with xanthan gum IPN Hydrogels occurs maximum at neutral pH, followed by acidic pH and least in neutral pH, whereas in case of poly(HEMA) the maximum swelling is inacidic, than neutral and than basic.So presence of xanthan gum as IPN in poly(HEMA) changed the pH sensitivity, so initially while we find that HEMA as hydrogel cannot be so useful in colonic drug delivery but the xanthan gum improves that swelling property of HEMA gel which is how a better hydrogel can be used in colonic drug delivery.

4.2.2 Isabgol hydrogel (at 37°C)

a)



b)



c)

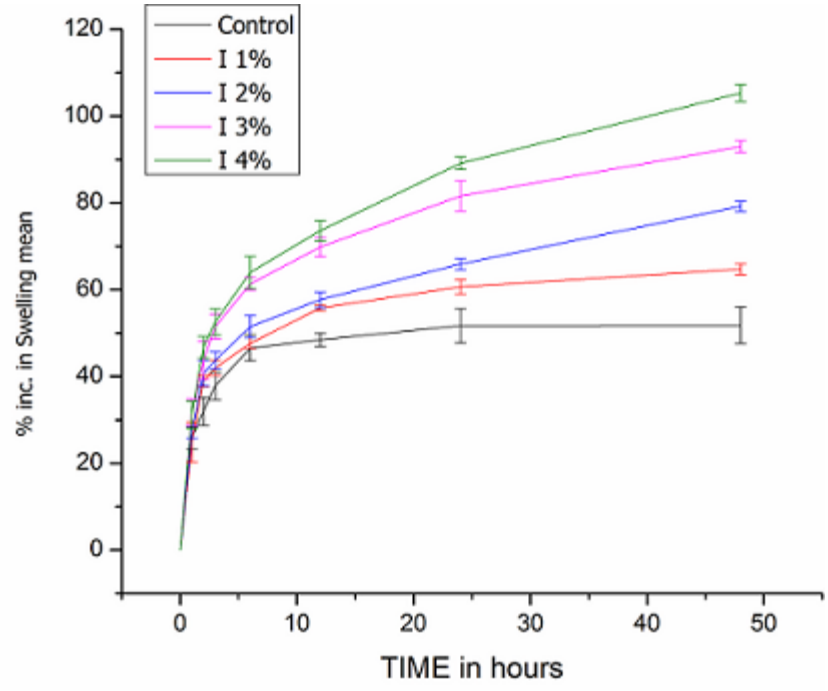


Figure 4.2 : Swelling study plot of Isabgol hydrogel at a) pH 1.2, b) pH 7, c) pH 9 at different concentration

Figure 4.2 a) shows the following results of the swelling study ofIsabgol hydrogel at pH 1.2 :

- The maximum swelling is shown by isabgol hydrogel of 1% isabgol w/v with a % increase in swelling mean of 160%.
- The second highest swelling is shown by isabgol hydrogel of 4% isabgol w/v conc. with a % increase in swelling mean of 150%.
- The third highest swelling is shown by isabgol hydrogel of 3% isabgol w/v conc.with a % increase in swelling mean of 140%
- The least swelling effect is shown by isabgol hydrogel of 4% isabgol w/v conc. with a % increase in swelling mean of 115% .
- The control is showing a % swelling mean of 80%
- Swelling effect : I 1%> I 2% > I 3% > I 4% > control.

Similar kind of result was found in isabgol Hydrogels as was found in xanthan gum hydrogels in pH 1.2 that isabgol IPN hydrogles swell more at acidic pH than Hydrogels of poly(HEMA). It also shows that isabgol Hydrogels can be used for colonic drug delivery at more acidic condition and isabgol hydrogles are more acidic sensitive.

Similarly figure 4.2 b) and c) reveals that :

- At pH 7
  - ❖ The maximum swelling is shown by isabgol hydrogel of 4% isabgol w/v conc.with a % increase in swelling mean of 180%.
  - ❖ The second highest swelling effect isshown by isabgol hydrogel of 3% isabgol w/v conc.with a % increase in swelling mean of 171%.
  - ❖ The third highest swelling effect is shown by isabgol hydroge of 2% isabgol w/v conc. with a % increase in swelling mean of 162%.
  - ❖ The least swelling effect is shown by isabgol hydrogel of 1% isabgol w/v conc. With a % increase in swelling mean of 151%.
  - ❖ The control is showing a % swelling mean of 60%
  - ❖ Swelling effect : I 4% > I 3% > I 2% > I 1 % > control.
  - ❖ Here also similar kind of result was found as was found in xanthan gum hydrogels in pH 7. .
- And at pH 9
  - ❖ The maximum swelling is shown by isabgol hydrogel of 4% isabgol w/v conc.with a % increase in swelling mean of 100%.
  - ❖ The second highest swelling effect isshown by isabgol hydrogel of 3% isabgol w/v conc.with a % increase in swelling mean of 93%.

- ❖ The third highest swelling effect is shown by isabgol hydroge of 2% isabgol w/v conc. with a % increase in swelling mean of 71%.
- ❖ The least swelling effect is shown by isabgol hydrogel of 1% isabgol w/v conc. With a % increase in swelling mean of 59%.
- ❖ The control is showing a % swelling mean of 51%.
- ❖ Swelling effect : I 4% > I 3% > I 2% > I 1 % > control.

The swelling study of both the hydrogels (i.e xanthan gum and isabgol) provides the information that, as concentration of polymer increases , its swelling effect increases. The result shows that swelling effect is far better, if hydrogels were prepared from xanthan gum or isabgol with HEMA instead of preparing hydrogels only with HEMA. Same trend was noticed in isabgol hydrogel in pH 9 as was seen in xanthan gum hydrogel.

4.3Moisture absortion study

4.3.1 Xanthan gum hydrogel (at 37°C)

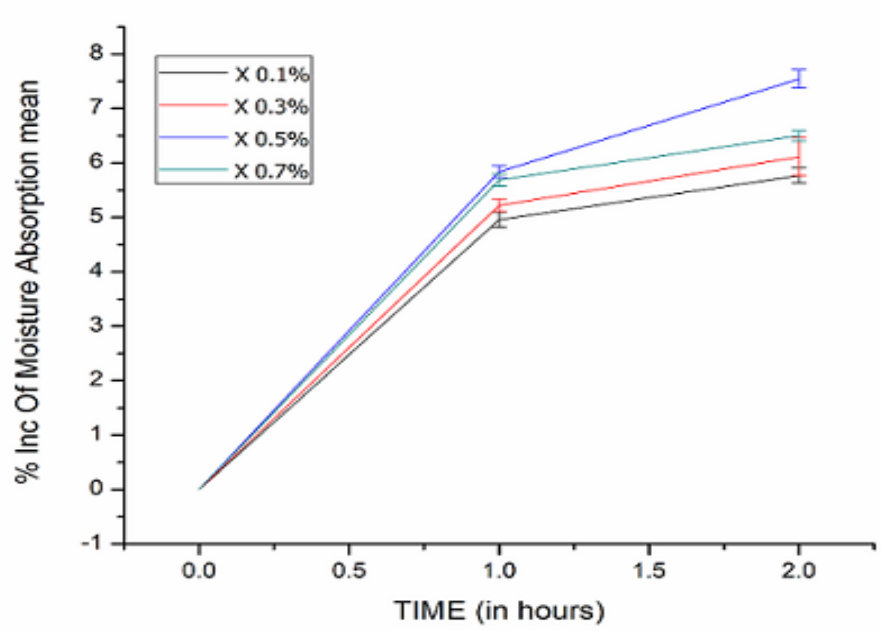


Figure 4.3 : Moisture absorption study of Xanthan gum hydrogel at different concentration

The above figure shows the moisture effect on the hydrogel of xanthan gum. The result reveals that at concentration of 0.5 % , effect of moisture is highest on the hydrogel. However, at concentration of 0.7 % , the effect of moisture is more than on 0.3 % and 0.1 % concentration.

4.3.2 Isabgol hydrogel (at 37^{o}C)

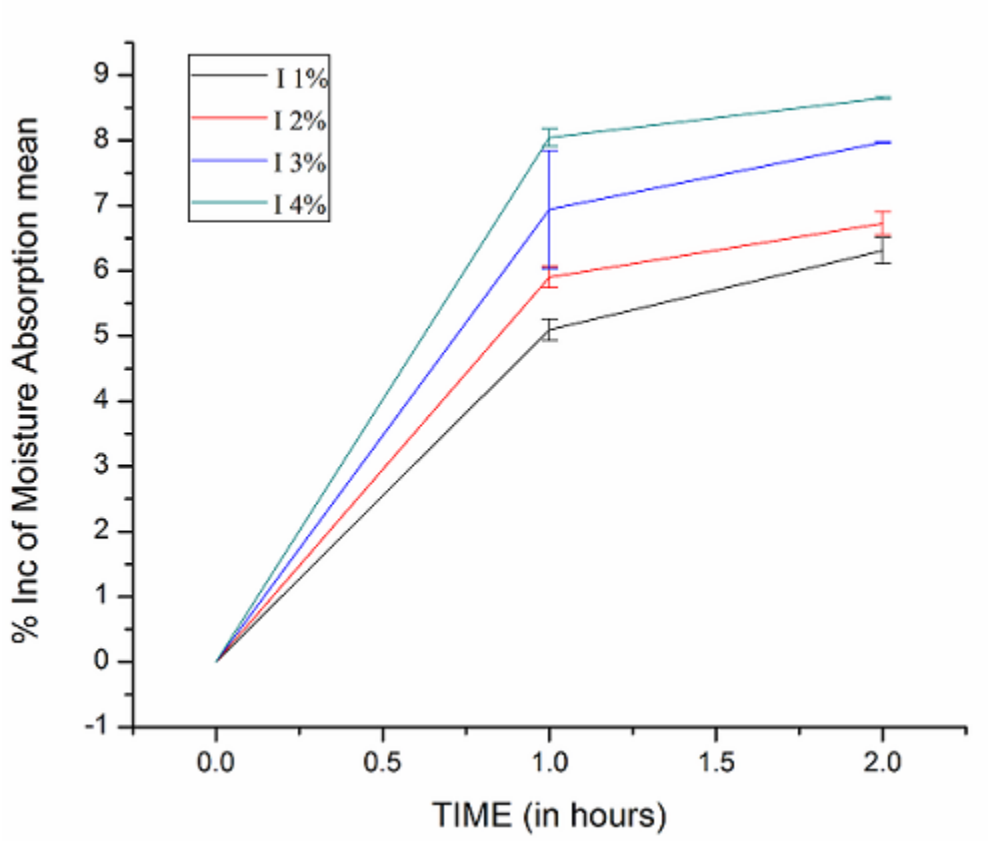


Figure4.4: Moistureabsorption study of Isabgol hydrogel at different concentration

4.4 Hemocompatibility Test

Positive Control : 1.71

Negative Control : 0.13

Table No. 2 : % Hemolysis at different concentration of xanthan gum IPN

Conc. of xanthan gum in hydrogels in w/v	**% Hemolysis**
X-0.1%	8.352
X-0.3%	7.895
X-0.5%	9.330
X-0.7%	9.699

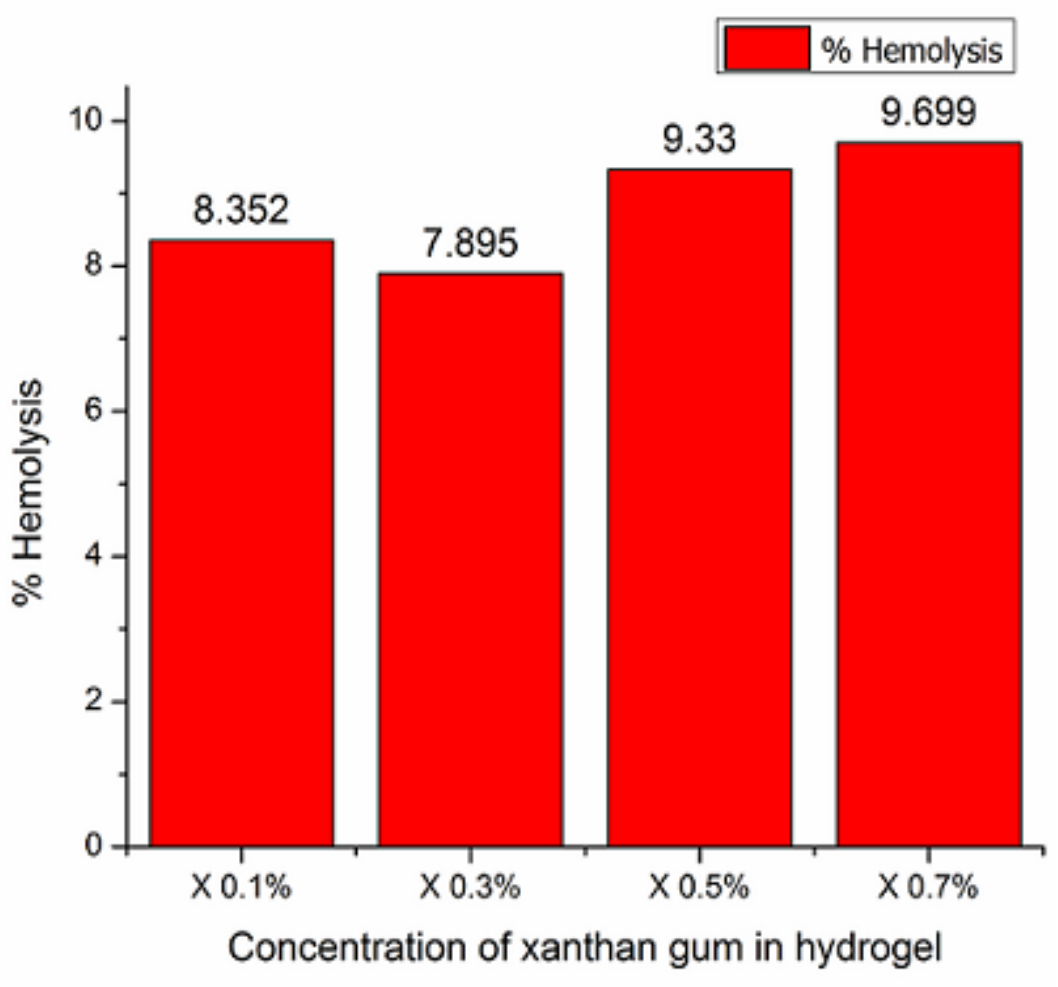


Figure4.5 : % Hemolysis in xanthan gum hydrogel

Table No. 3 : % Hemolysis at different concentration of isabgol IPN

Conc. of Isabgol in hydrogel in w/v	**% Hemolysis**
I-1%	7.416
I-2.5%	7.656
I-5%	8.852
I-7%	8.612

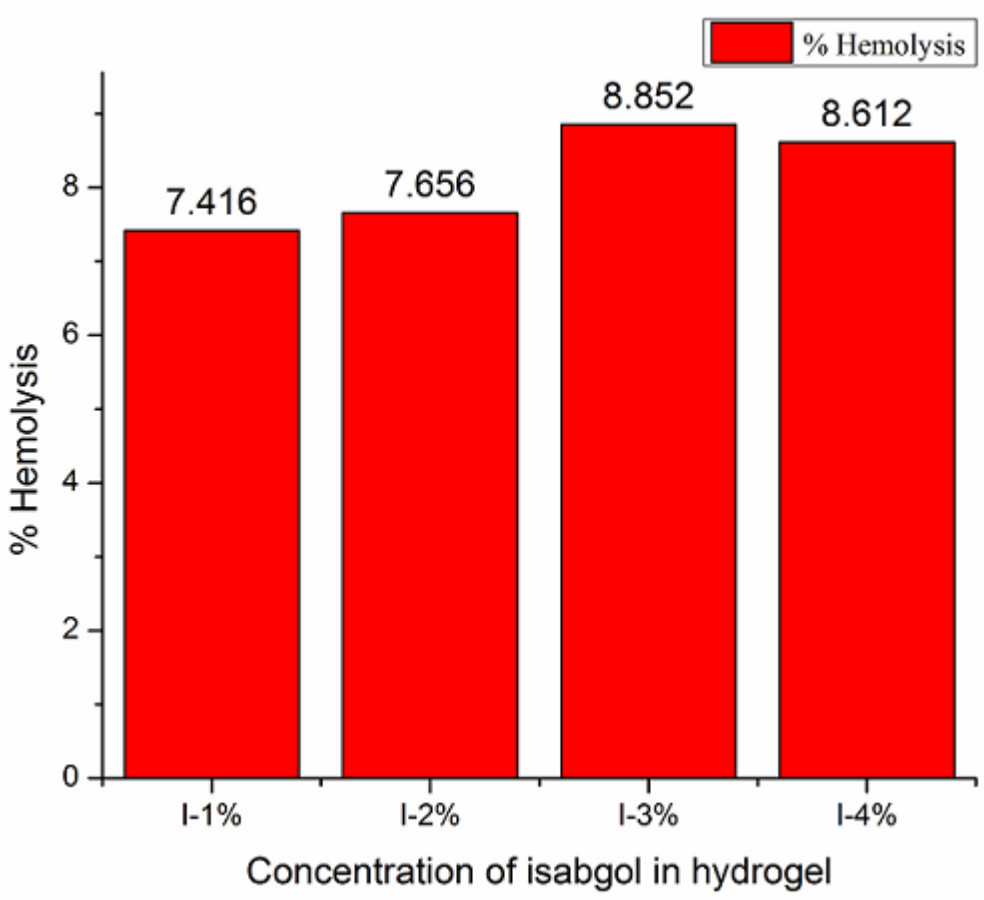
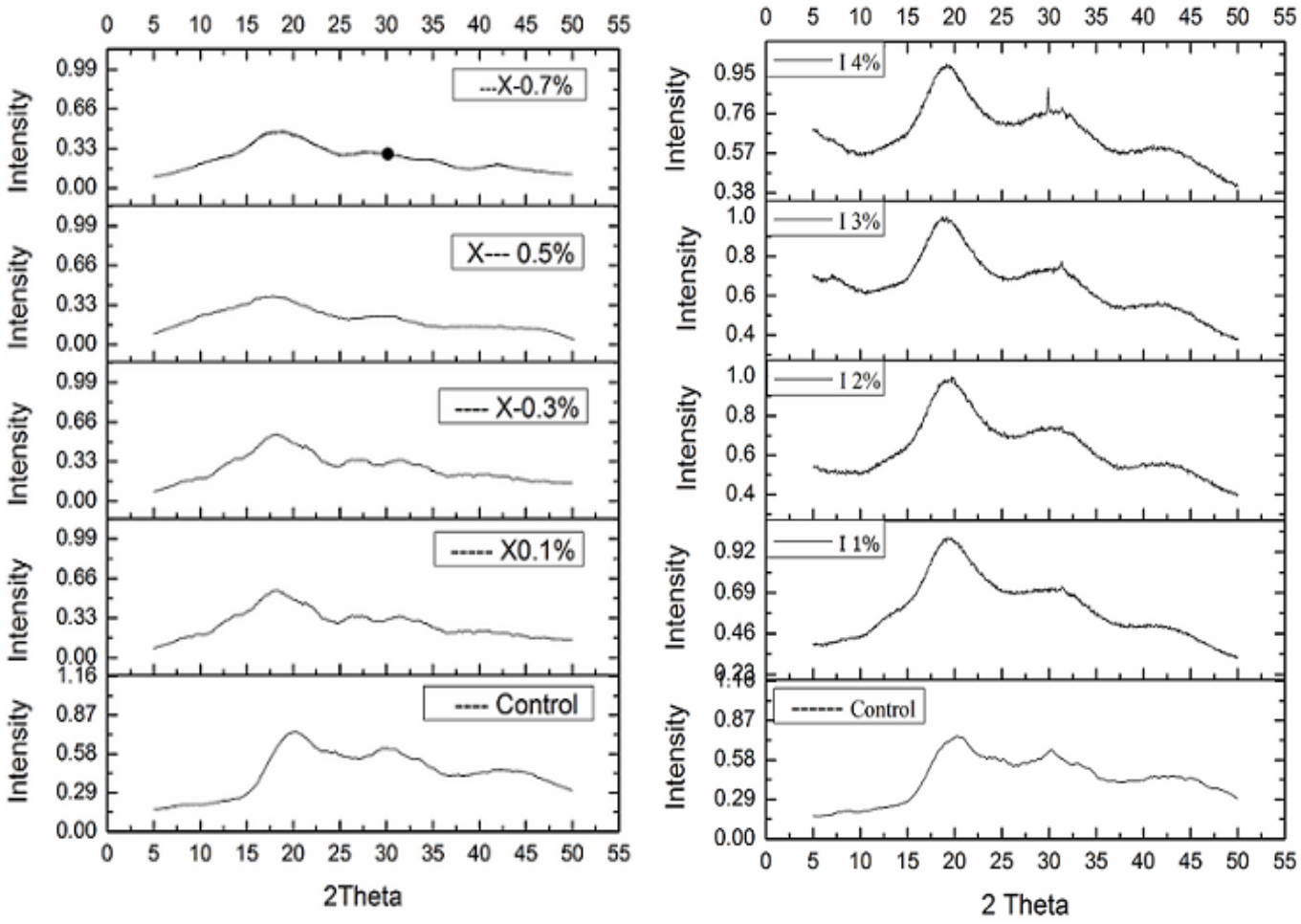


Figure 4.6 : % Hemolysis in isabgol hydrogel

From the graph of hemolysis shown in figure 4.5 and 4.6 of xanthan gum and isabgol hydrogel respectively, it is observed that break down of red blood cells is less in isabgol hydrogels compared to xanthan gum hydrogel. It is important to note that if % hemolysis is less than 5, then the biomaterial is most acceptable by the body and if its range is between 5 to 10, the material can me just acceptable by the body, but it shoud not cross beyond 10, otherwise it is harmful to the body, so it is concluded that % hemolysis is falling between 5 to 10 % which shows these hydrogels are moderately hemocompatible and hence are acceptable material.

4.5 X-Ray Diffraction Spectroscopy (XRD)

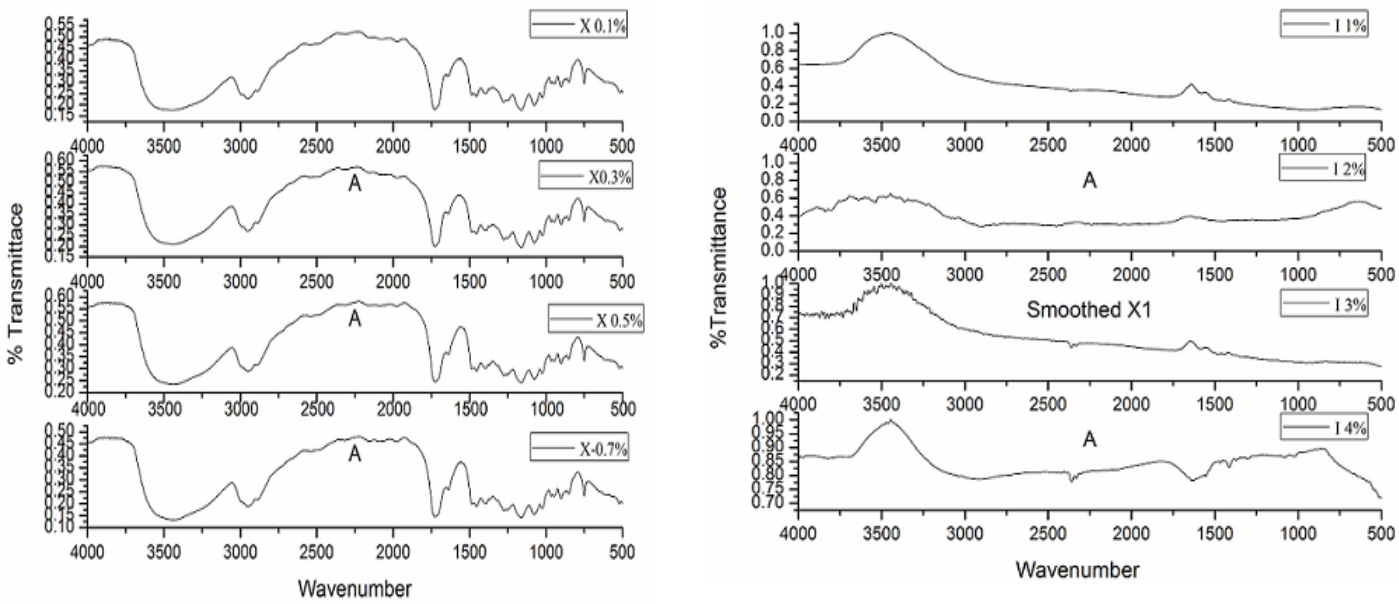


a) **b)**

Figure 4.7: XRD plot of different concentrations of a) Xanthan gum b) Isabgol Hydrogels

From the XRD plot of the xanthan gum and isabgol hydrogel shown above, it can concludedthat maximum peak obtained between 15 to 20 degree angle. The peaks broadening of hydrogels sample show the non uniform microstrain, this may be because of higher concentrations of polysaccharides. Hydrogels made of poly(HEMA) have more non uniform microstrains compared to xanthan and isabgol hydrogels.

4.6 Fourier Tranform Infrared Spectroscopy (FTIR)



a) **b)**

Figure 4.8: FTIR of a) Xanthan gum b) Isabgol hydrogels

The FTIR images of xanthan gum and isabgol hydrogel shown above reveals that,% transmission absorption is more in xanthan gum hydrogels compared to isabgol hydrogels.

FTIR of HEMA

Stretching of Ester group has a max absorption of light in the wavenumber between 1735 cm^{-1} to 1153 cm^{-1}.

CH stretching of CH_3 group has max absorption of light in the wavenumber between 2952cm^{-1}

FTIR of Xanthan Gum

OH group of xanthan gum has max absorption of light in the wavenumber of 3450 cm^{-1}.

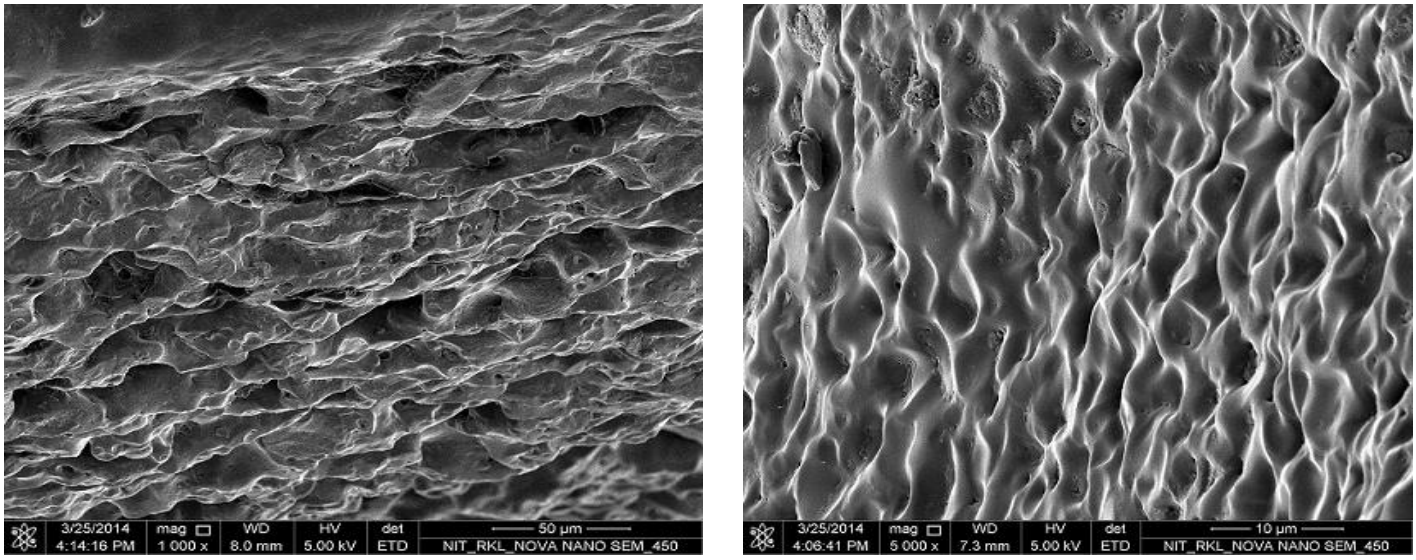
FTIR of Isabgol

OH stretching has max absorption of light in the wavenumber of 3401 cm^{-1}.

CH stretching of alkaline group has max absorption of light in the wavenumber of 2926 $cm^{-1.}$

C-O-C stretching of ester group has max absorption of light in the wavenumber of 1050 cm^{-1}.

4.7 Field Emission – Scanning Electron Microscopy (FESEM)



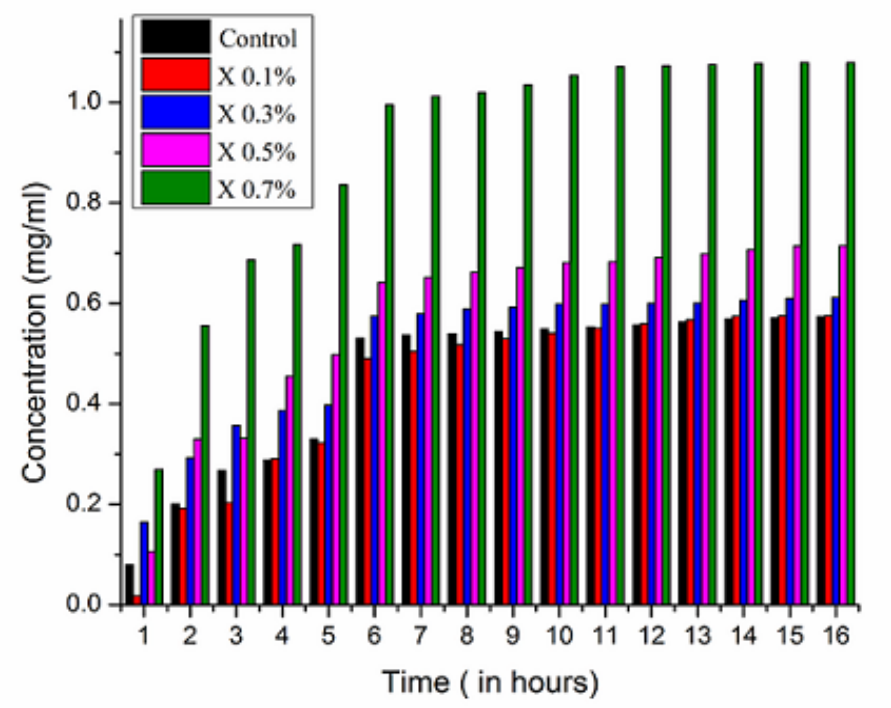
a) **b)**

Figure 4.9 : FESEM images of a) Xanthan gum b) Isabgol hydrogels

The FESEM images of the hydrogels show above reveals that xanthan gum hydrogels have sharp edges and isabgol is having more roundings.

4.8 Drug Release Kinetics

a)



b)

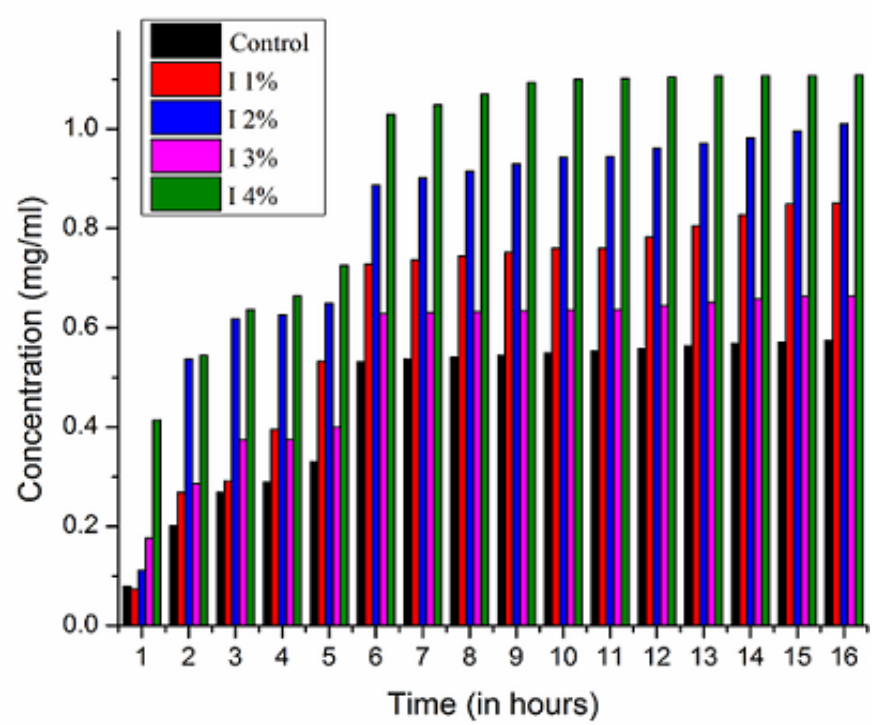


Figure 4.10 : Drug release images of a) Xanthan gum b) Isabgol hydrogels

Figure 4.10 a) and b) shows that max curcumin absorption took place at pH 6.8 which is the pH value available in colon. After conducting drug kinetics test for 16 hours in both polymer based hydrogels it was found that 1mg/ml curcumin was released by the highest conc. of xanthan gum hydrogel and 1mg/ml curcumin was released by highest conc. of isabgol hydrogels.

CHAPTER 5 : CONCLUSION AND FUTURE PROSPECTS

The effect of all the tests done on hydrogels of xanthan gum and isabgol shows that interpenetrating hydrogels show better performance in colon drug delivery compared to hydrogels made of single polymer. Since xanthan gum and isabgol are natural polymers from the family of polysaccharides, they can swell, incorporate and release drug better compared to hydrogles of only HEMA.

The other experiments required to be performed are :

- Biodegradability in presence of enzymes
- Biodegradability in presence of microorganisms
- Biocompatibility
- Mechanical Tests
- Drug release kinetics using some other drugs in the hydrogels

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