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Formulation physical characterization and in-vitro release studies of novel polymer composites for chronic wound healing

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ABSTRACT

The composite sponges were prepared by incorporating 5% of povidone-iodine into polymers like Gelatin, Acacia, Hydroxy Propyl Methyl Cellulose (HPMC) and Chitosan the excipients were used in different ratios and two different methods was used to prepare formulations. Prepared formulations were evaluated for drug excipient interactions, by Fourier Transform Infrared Spectroscopy (FT-IR) which elucidate there is no interactions between constituents used in formulation, morphology of the selected formulations were studied by Scanning Electron Microscopy (SEM) the results revealed that fine pores and smooth surfaces on the sponges and these sponges were evaluated for Physical Properties like Sponge Hydration index which showed formulation which were freeze dried formulations possess good hydration index. pH of sponge was found in the range of 5.5 to 5.9, Drug content was found to be 24.89mg and 24.93mg respectively. And In -Vitro Drug Release studies showed a percentage drug release of 75.15% and 76.26% respectively initially burst release was observed for both F1 and F3 formulations. Stability study for 3 months indicated that formulations were stable and results were comparable with initial results.

Key words: Povidone Iodine, Gelatin, HPMC, Chitosan, Sponges for Wound healing Antimicrobial Activity.

INTRODUCTION

The objective of the study is to develop novel polymer composite to achieve wound contraction with the help of exogenous wound healing substances.

For effective wound healing conditions like warm and moist environment which is widely accepted for rapid wound healing and most modern wound care products are designed to provide these conditions. Hydroxy Propyl Methyl Cellulose (HPMC) is semisynthetic polymer which is capable of forming in-situ hydrogel by temperature induced mechanisms. HPMC possess excellent film forming property and superior tensile strength [1].Have tried to develop topical formulations of rhVEGF gel and for preparation, different cellulose derivatives such as HPMC, MC, HEC and CMC were considered. Also due to its good swelling behavior, it enhances wound healing by maintaining moist environment.

The skin pH was observed to be acidic in nature in the range of 4 – 6; this becomes alkaline with wounds thus delays wound healing [2, 3]. It is found that acidic pH promotes wound healing.

The surface pH of wound and level of Protease in wound always plays a major role in wound contraction pH on the surface of the wounds was found to be ranging from 7 – 8.9 wounds with alkaline pH shows lower healing rate [4,5,6].

The healing rate of chronic wound is high with prolonged chemical acidification acidic pH promotes wound healing activity than alkaline pH, pH turns to neutral and proceed towards acidic during wound healing [5, 7].acidic pH was observed on the wounds during healing activity. It also observed that wound pH found to increase with stage 1, stage 2 and stage 3 ulcers respectively.

Hence the prime objective of this study is to achieve lower (acidic) pH on wound surface by using two acid soluble polymers, gelatin and chitosan. High Protease level with elevated pH can be minimized by making the acidic environment through these acid soluble polymers. The wound healing activity of gelatin is by its nature of stimulating human monocytes to generate high levels of tumour necrosis factors such as an interleukin[6, 8].Production of TNF at wound site results in a pro-inflammatory stimulus is advantageous to wound healing. Hence gelatin is also considered for the study.

Antibacterial agents in combination with natural polymer can give better wound healing environment for wound retrenchment by providing Static effect and also prevent additional infectivity. In this study, proved inherent anti-microbial properties of chitosan are used to achieve and deliver extrinsic antimicrobial agents to wounds and burns through controlled manner [9]. Chitosan is known for its immunostimulatory activities and anticoagulant properties, good antibacterial agent and antifungal action it is used as promoter of wound healing in surgeries, [10,11,12].Topical broad spectrum antiseptic Povidone-iodine which is also incorporated into the formulations synergize anti- microbial effect by Controlling the normal inflammatory process and by serving as a signal suppressor that down-regulates specific enzymatic activities which are typically elevated during inflammation [13,14,15].Additionally PVP –Iodine combination have proven effectiveness in preventing and overcoming microbial infections also with respect to severe ulceration[16,17].PVP – Iodine which is official in USP and EP.

Acetic acid 5% solutions has been widely used in an attempt to reduce the pH.

However, acetic acid lowers pH for only one hour after which it returns to pretreatment levels [18].

These polymer and drug composites can be a better wound healing tools for the acute as well as the chronic wound.

MATERIALS AND METHODS

2.1 MATERIALS:

- Drug and Polymer Used – Povidone Iodine, Gelatin, Chitosan, HPMC, Acacia
- Instruments Used – Hot air oven, lyophillizer, dissolution apparatus, magnetic stirrer, spectrophotometer (UV), Fourier Transform Infrared Spectroscopy (FT-IR), Scanning Electron Microscopy (SEM)-Hitachi, S-2400.

Table no.1 Compositions of formulations prepared by method-1.

CONSTITUENTS	F1	F2	F3	F4	F5	F6	F7	F8	F9
GELATIN	1g	1g	1g	0.6g	0.5g	0.4g	0.4g	0.5g	0.6g
CHITOSAN	0.6g	0.5g	0.4g	1g	1g	1g	0.6g	0.5g	0.4g
HPMC	0.4g	0.5g	0.6g	0.4g	0.5g	0.6g	1g	1g	1g

2.2 METHOD OF PREPARATION

PREPARATION OF WOUND EXUDATE ABSORBING SPONGE:

The formulations were prepared by dissolving Gelatin in 5 ml of 0.5M acetic acid, Chitosan and HPMC was dissolved in 15 ml distilled water. Two solutions with dissolved polymers were mixed by stirring, calculated amount of 5% Povidone iodine was also added to the mixture. Air bubbles were removed by vacuum pressure and the mixture was transferred into 35 mm petridish. Petridish with mixture was freeze dried for 48 hours Overnight in-

40°C. Wound exudates were collected and placed under dry place. Table no.1 describes the compositions of formulations prepared by method 1. The formulation weighs about 2gms with the given ratio

METHOD-2
Acacia, Chitosan and HPMC were dissolved in distilled water. Air bubbles were removed by vacuum pressure, calculated amount of 5% Povidone iodine was also added to the mixture. The mixture was transferred into 35mm petridish. Petridish with mixure was stored overnight under -40°C, freeze drying was performed. Wound exudates were collected and placed under dry place.

Table No: 2 Compositions of formulations prepared by method-2.

CONSTITUENTS	F1	F2	F3	F4	F5	F6	F7	F8	F9
ACACIA	1g	1g	1g	0.6g	0.5g	0.4g	0.4g	0.5g	0.6g
CHITOSAN	0.6g	0.5g	0.4g	1g	1g	1g	0.6g	0.5g	0.4g
HPMC	0.4g	0.5g	0.6g	0.4g	0.5g	0.6g	1g	1g	1g

3. CHARACTERIZATION

3.1 PHYSICAL PROPERTIES

After collection of samples from the freeze dryers, all preparations were physically examined for color, odour, and thickness. Thickness of polymer composite films were determined by digital vernier caliper. Vernier caliper was positioned at three different areas of each formulation and to determine thickness on the entire area and average of triplicate value was calculated.

3.2 SPONGE HYDRATION INDEX.

The sponges were separated and about 0.65g of each formulation was taken to study sponge hydration index. The sponges were immersed in 50ml of pH-7.4 solution for about 2hrs. After 2hrs the formulations were taken out and the weight was noted. With the difference in weight the amount of solution absorbed by the formulation was calculated by using formula [20].

$$\% \text{ water uptake} = \frac{W_{AT} - W_{BO}}{W_{BO}}$$

WAT – weight of film after hydration at time‘t’
WBT – weight of film before hydration at time ‘0’

3.3 pH OF WOUND SPONGE

Accurately measured quantity 0.500 g of selected formulations were sonicated and dispersed with distilled water pH 7.0. The resulting solutions were centrifuged at 15,000 rpm for 15 minutes to remove the supernatant and the pH difference between distilled water and resulting supernatant was determined.

3.4 DRUG – POLYMER INTERACTION:

The drug polymer interaction was studied through FT-IR study. The procedure is as follows: The KBr pellet with drug, excipients and formulations were prepared and spectra were recorded in the wavelength range of 4000 and 400 cm–1. The spectra obtained for drug, excipients, and formulations were compared.

3.5 DRUG CONTENT

Accurately measured quantity 0.500 g of selected formulation was sonicated and dispersed with distilled water of pH 7.0. The resulting solution was centrifuged at 15,000 rpm for 15 minutes to remove the supernatant and the drug concentration in resulting supernatant was determined by using UV-Spectrophotometer at 265nm.

3.6 IN-VITRO DRUG RELEASE. [21].

In vitro drug release was performed by immersing the sponges in 250ml beaker filled with 100 ml 7.4 pH.the solutions and paddle at 50 rpm was placed at the right position, with 37°C temperature. The samples were collected

at predefined intervals 5, 10, 15, 30, 45, 60 min for the 1st hr and later the samples were collected at time interval of 1hr up to 8hours. The absorbances of collected samples were measured by UV-spectrophotometer at 265nm.

3.7 EVALUATION OF ANTIMICROBIAL ACTIVITY.

Antimicrobial activity of Chitosan and composite sponge were evaluated by agar diffusion method using pseudomonas aeruginosa. Zone of distribution was identified by placing 2 × 2 cm of sponge over the preinoculated solidified agar medium in a petri dish followed by incubation at 37°c over a period of 24 hours [20].

3.8 SCANNING ELECTRON MICROSCOPY (SEM)

Morphology character of the polymer composite sponge was examined by scanning electron microscope (Hitachi, S-2400). Polymer composite sponge was placed on aluminium stubs and coated with gold (15nm) using Electrodag silver conducting point and sputter coater respectively. Then the prepared samples were examined for surface characters.

3.9 STABILITY STUDIES

Based on the hydration index formulation F3 was selected for stability study. Selected formulation F3 was placed in the stability chamber at 45°C, with 75% relative humidity for 3 months. Periodically (0, 1, 2 and 3 months) samples were taken and analyzed for physical properties like colour, odour, pH, drug content and in-*vitro* drug release.

RESULTS AND DISCUSSION

By following method 1 Nine different formulations were prepared employing three different polymers like Gelatin, Chitosan and HPMC. By following method 2 nine formulations were prepared with three different polymers of Acacia, Chitosan and HPMC. A concentration of 5% povidone iodine was incorporated in all the formulations.

Table No 1 and 2 shows the different polymer ratios and compositions of formulations prepared by method 1 and method 2.

4.1 PHYSICAL PROPERTIES:

Colour and odour of prepared formulation was found to be pale yellow and acidic respectively. The pale yellow colour of sponges shall be attributed to the presence of natural polymer gelatin and Chitosan. Acidic odour was due to the utilization of 0.5m acetic acid in the formulation. After complete freeze drying, the thickness of the sponges was determined by using vernier caliper. Table no: 3 shows thickness values of all formulations prepared. Gelatin concentration plays a prime role in thickness of sponges' formulations with high concentration of gelatin shows high thickness whereas the other polymer exhibits significant shrink after freeze drying.

TABLE NO: 3 Thickness of formulations prepared.

SPONGES	F1	F2	F3	F4	F5	F6	F7	F8	F9
THICKNESS (MM)	3.7	3.8	3.7	3.5	3.4	3.4	3.6	3.5	3.5

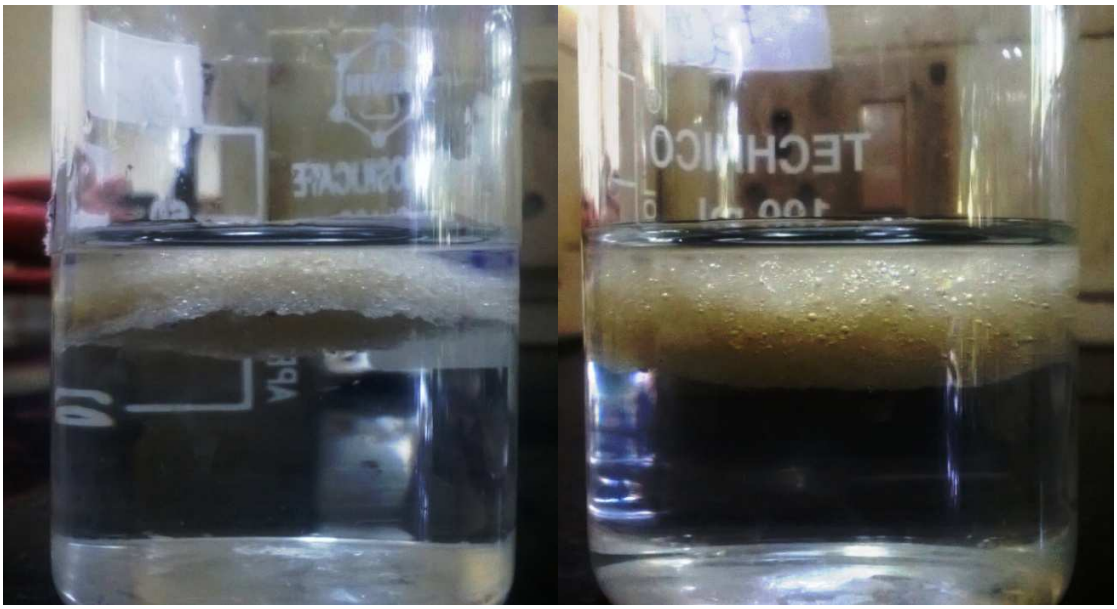
4.2 SPONGE HYDRATION INDEX

Water uptake of all formulation sponges was determined by using difference in weight before and after water uptake. Percentage of water uptake was calculated and tabulated in table 4. Sponge prepared by using method 1 is having good water up take capacity than formulations prepared by method 2. Highest amount of water absorption was found in formulations F1 and F3 which was selected from the method 1 for further analysis. Both F1 and F3 formulation possess same concentration of gelatin, maximum concentration of chitosan and HPMC respectively. Due to the high water absorbing nature and tendency for rapid disintegration of acacia could not able to maintain the structural integrity of sponge when exposed to the watery environment. Chitosan and HPMC able to form Gel like environment of sponge produced by absorbing the wound exudates. Chitosan also can maintain the structural integrity of sponge due to its slow swelling,

TABLE NO: 4 Hydration Index of prepared formulations.

SPONGES	F1	F2	F3	F4	F5	F6	F7	F8	F9
HYDRATION INDEX (%)	201.6	207.6	253.6	201.6	169.2	161.5	123	138	164

FIG: 1 Figure 01 illustrates the hydration of polymers.



4.3 pH OF COMPOSITES SPONGE:

pH of all the sponges were measured after the complete disintegration of polymers in pH 7.0 distilled water. Results were tabulated in the table number 5. pH of distilled water significantly changes for all the sponges due to gelatin and chitosan which was dissolved 0.5M acetic acid initially.

Equal quantity of acetic acid was used to dissolve polymers therefore significant changes in pH was observed but not between the sponges.

TABLE NO.5 Shows pH of formulations prepared.

SPONGES	F1	F2	F3	F4	F5	F6	F7	F8	F9
pH	5.8	5.9	5.5	5.8	5.	5.6	5.7	5.9	5.6

4.4 DRUG POLYMER INTERACTIONS:

Interaction between povidone iodine and polymer was studied by the KBR pellets technique. Figure 2-6 shows the FTIR spectrum of gelatin, chitosan, HPMC, povidone iodine and F3 formulation respectively. F3 sponges spectrum (Fig - 6 spectra) shows intermolecular hydrogen bond peak at 3521cm^{-1}, secondary amide peak at 1421 cm^{-1}, cellulose peak at 1093cm^{-1} and OH associated peak at 2889cm^{-1}. From spectrum obtained it was concluded that there was no significant interaction between drug and polymers due to intermolecular hydrogen bond peak for povidone iodine, secondary amide peak for gelatin, cellulose peak for chitosan and HPMC and OH associated peak for hydrophilic HPMC.

FIG: 2 FTIR SPECTRUM- GELATIN.

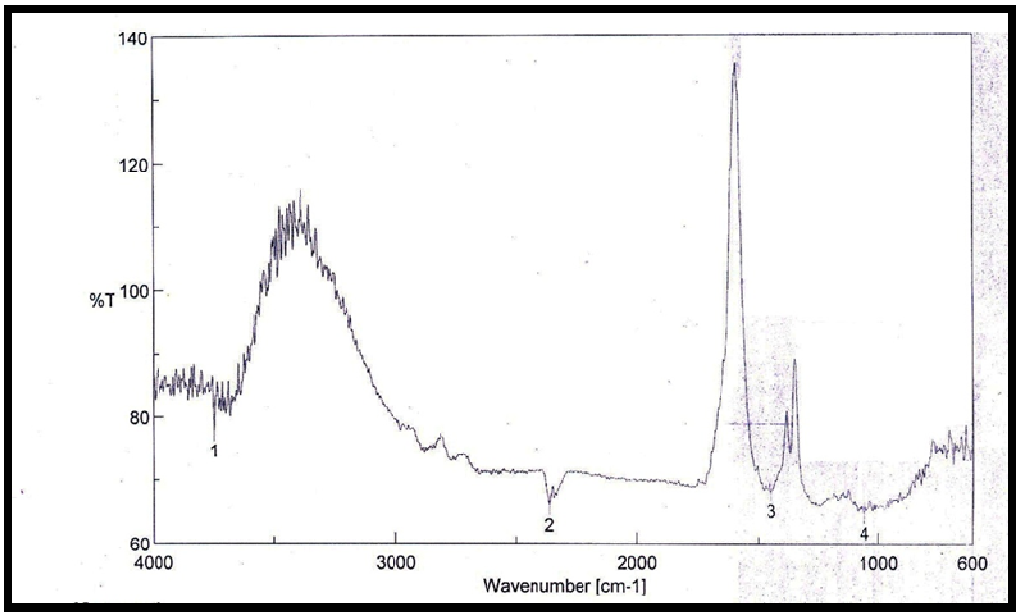


FIG: 3 FTIR SPECTRUM- CHITOSAN.

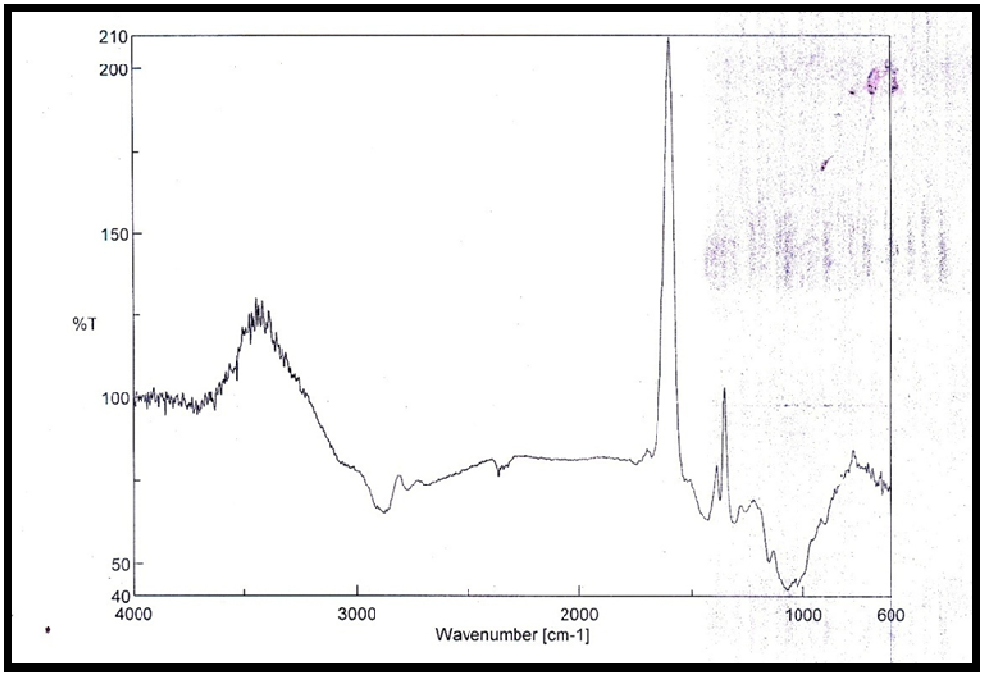


FIG: 4 FTIR SPECTRUM- HPMC.

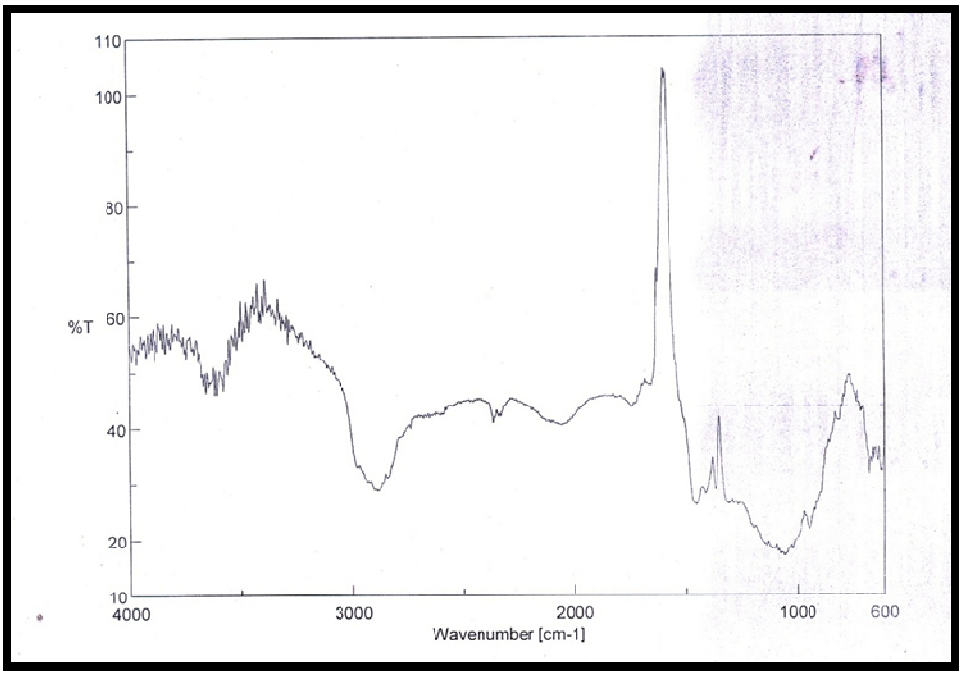


FIG: 5 FTIR SPECTRUM- POVIDONE IODINE.

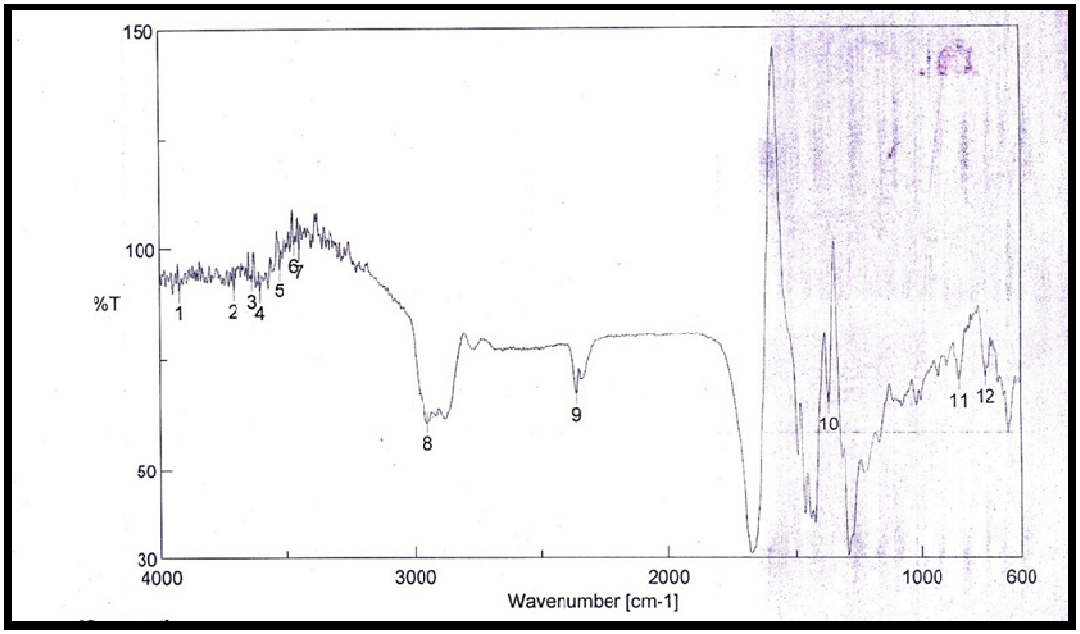
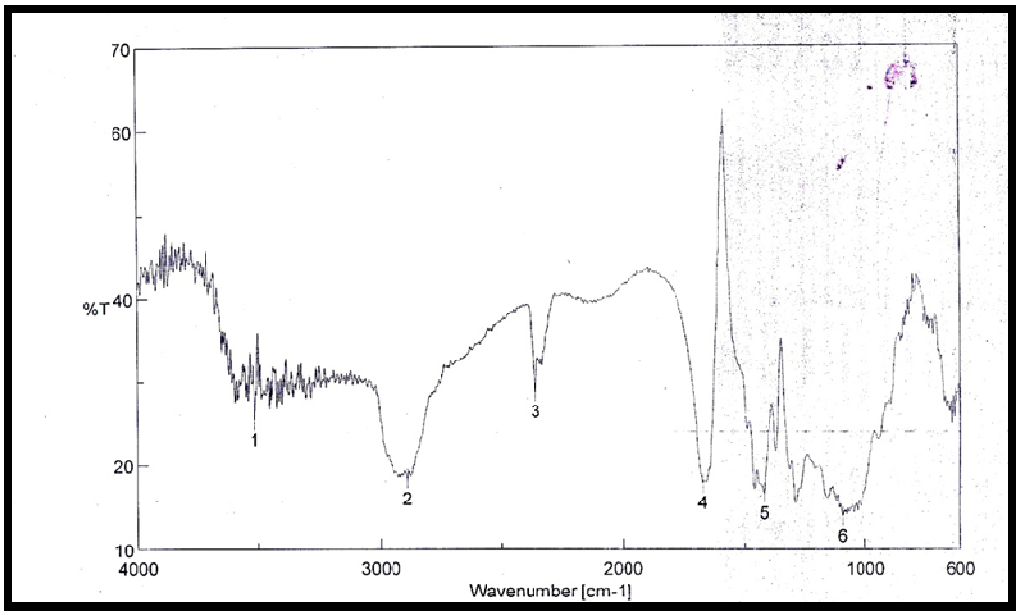


FIG: 6 FTIR SPECTRUM- F3 SPONGE



4.5 DRUG CONTENT (ASSAY):

F1 and F3 were selected from method1 based on hydration index. 0.500gm of F1 and F3 formulation was analyzed and drug content and drug content was 24.89mg and 24.93mg respectively. This shows no significant changes of drug content while formulating the sponges and also no interaction between the polymers and drug.

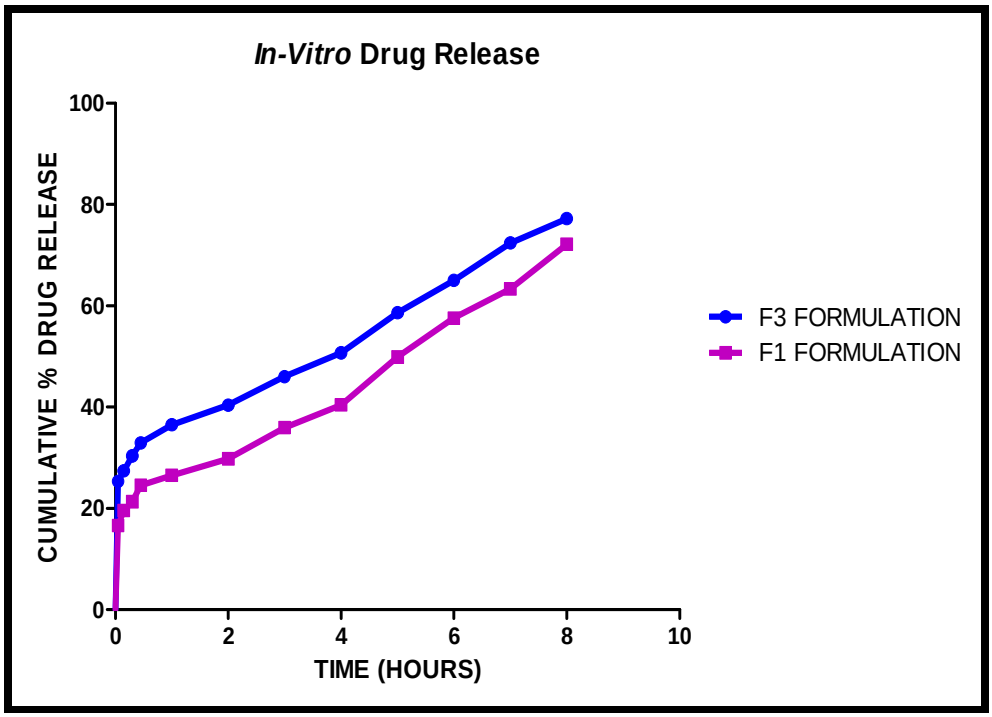
4.6 IN-VITRO DRUG RELEASE: [21].

In-vitro drug release study was performed for F1 and F3 formulations. The percentage of drug release was found to be 75.15% and 76.26% respectively. Initially burst release was observed for both F1 and F3 formulations due to fast diffusion of dissolution medium and rapid solubility of drug. After certain time the release was delayed which might be due to the formation of rate limiting membrane around each particles which retards the drug release after certain duration. Due to the entanglement of drug in polymers it showed extended release over a period of time [22]. Table 7 and Table Depicts the release profile of formulations F3 and F1. Figure 7 shows the cumulative drug release.

Table No: 7 Dissolution profile for formulation F3

Time (hours)	Cumulative Percentage Drug Release – F3	Cumulative Percentage Drug Release – F1
0.05	25.34	16.64
0.15	27.42	19.56
0.30	30.37	21.31
0.45	32.95	24.59
1	36.52	26.55
2	40.37	29.77
3	46.03	35.96
4	50.72	40.45
5	58.62	49.90
6	65.04	57.60
7	72.42	63.33
8	77.26	72.15

FIGURE: 7 CUMULATIVE DRUG RELEASE OF FORMULATIONS F3 AND FI



4.7 SCANNING ELECTRON MICROSCOPY (SEM):

Scanning electron microscopy was performed for formulation F3. It revealed that Very fine pores and smooth surface was observed on the surface of sponge which may be due to 48 hours lyophilisation of formulation, movement of water molecules from the interface to surface might create Pores. These pores were completely filled by the Watery external environment and this would be a reason for fast dissolution and diffusion of drug to the external environment. Presence of smooth surface on sponge leads to good adhesion to wound surface and promote the normal healing progress.

4.8 STABILITY STUDIES

Stability study for formulations was carried out and results were tabulated in table no: 8. The colour and odour of F3 sponges was initially pale yellow and acidic. Pale yellow colour due to presence of gelatin, and chitosan in formulation leads to acidic odour due to the 0.5m acetic acid which was used initially for the formulation development. pH of sponges did not altered even at end of 3 months. Drug content and *in-vitro* drug release of formulation F3 was not changed in stability period. Therefore the F3 polymer composites sponge was stable and can be able to store in dry place.

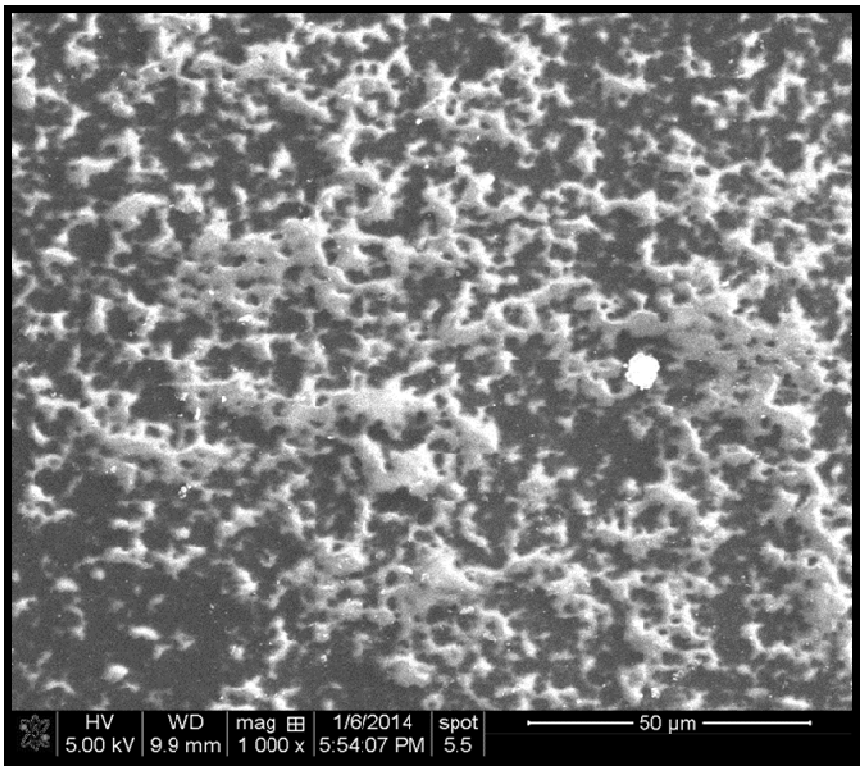
TABLE: 8 STABILITY DATA OF FORMULATION F3

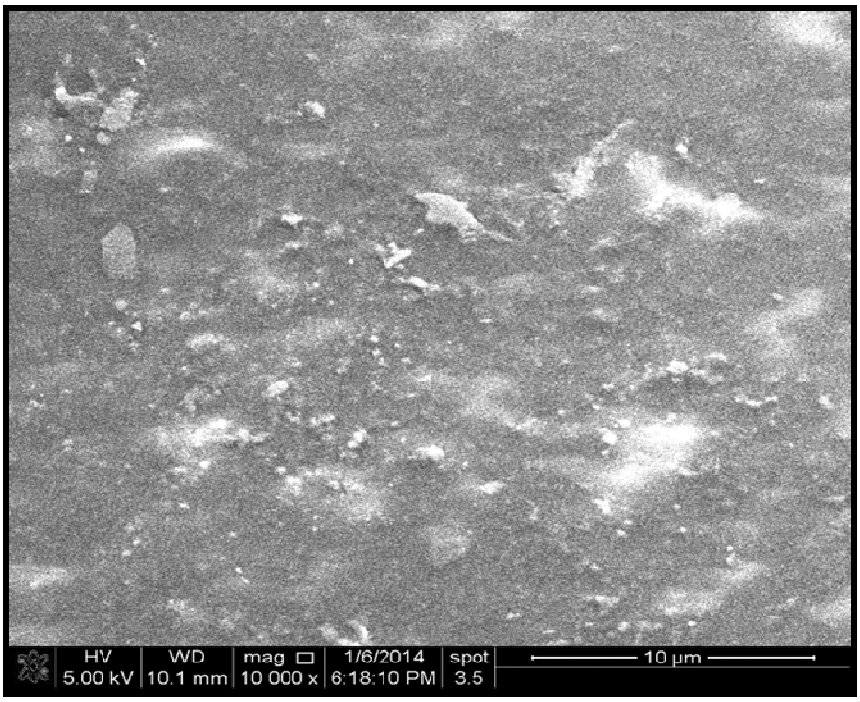
TIME (MONTHS)	COLOUR	ODOUR	pH of sponge	Drug content (Mg)	% Drug release upto 8 hours
0	Pale yellow	Acidic odour	5.8	24.93	77.26
1	Pale yellow	Acidic odour	5.7	24.89	77.18
2	Pale yellow	Acidic odour	5.7	24.78	76.92
3	Pale yellow	Acidic odour	5.7	24.81	76.98

4.9 Antimicrobial activity

The obtained results were evaluated and the formulations were found to possess good antimicrobial activity, wich might be due to the presence of povidone iodine and chitosan in the formulation.

Figure: 8 SEM photograph of F3 formulation





CONCLUSION

The novel polymer composites of gelatin, chitosan and HPMC were successfully formulated as wound exudate absorbing sponges. Broad spectrum antibiotic povidone iodine was successfully incorporated in sponge. FTIR spectrum of the formulation shows there was no interaction between the drug and polymer and also concluded that its having the more stability even in the accelerated storage conditions. pH lowering capacity of sponge can make acidic environment around the wound, thus can give the high beneficial effect during wound contraction phase. Natural antibacterial property of chitosan also promotes antibacterial effect of povidone iodine. During wound contraction deficiency of extra cellular matrix can be compensated by gelatin due to its ability to inhibit the elevated protease level in chronic wound. Swelling and film forming polymer HPMC can give good bio-adhesion property by absorbing exudate from wound.

Therefore these formulations shall be considered highly safe and effective in chronic wounds like leprosy, diabetic foot ulcer and long-lasting accidental wounds etc further in-vivo studies required to prove safety, efficacy, and to evaluate and suitability of dosage form for intended use.

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