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**FORMULATION AND CHARACTERIZATION OF CROSS-LINKED HYDROGEL  
CONTAINING PIPERINE FOR WOUND HEALING**

**MALATHI S, PAVITHRA K AND SOMASUNDARAM I**

Department of Pharmaceutics, School of Pharmaceutical Sciences, Vels Institute of Science,  
Technology and Advance Studies (VISTAS), Pallavaram, Chennai - 600117, Tamil Nadu, India

**\*Corresponding Author: I. Somasundaram: E Mail: [somous0926@gmail.com](mailto:somous0926@gmail.com); Contact no: +91  
9176688058**

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**ABSTRACT**

**Objective:** The Aim of the present study is to formulate and evaluate the formulated cross-linked hydrogel containing piperine for the topical wound healing application.

**Methods:** The hydrogel containing piperine hydrogels were prepared by cross-linking method with the different concentrations of xanthan gum. Homogeneity, Rheological Studies, swelling studies, scanning electron microscopy (SEM) and *in vitro* drug release studies were carried out.

**Results:** The formulated hydrogel containing piperine was evaluated and based on the surface morphology, swelling studies, homogeneity, rheological studies and *in vitro* drug release studies the formulation F4 shows good results when compared to the other formulations.

**Conclusion:** Hydrogel system could play an important role in the treatment of skin injuries and infection. Cross-linked polymeric hydrogel membranes loaded with the piperine were synthesized successfully with an optimized process of cross linking which would act as new healing system for the wound healing burns with controlled release manner at wound site by preventing infection and increased healing efficiency through a contact.

**Key words: SEM, Piperine, Hydrogel**

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## INTRODUCTION

Hydrogels are a network of hydrophilic, water-insoluble polymer chains, sometimes found as a colloidal gel where water is the medium of dispersion [1]. Hydrogels are a cross-linked, three-dimensional polymer network which are usually formed by chemical or physical crosslinking that is smart enough to react to environmental stimulus fluctuations and swell or shrink accordingly. They are soft and rubbery in the swollen state, which resembles the living tissue with excellent biocompatibility. Hydrogels are cross-linked materials that absorb large amounts of water without being dissolved. Softness, smartness and the ability to store water make hydrogels unique materials. Hydrogels' ability to absorb water stems from hydrophilic functional groups attached to the polymer backbone while their dissolution resistance stems from cross-links between the network chains [2].

Hydrogel materials prepared by radiation cross linking technique from natural and synthetic hydrophilic polymers are suitable for burning/wound healing. Hydrogel dressing can cover the entire area of the wound with its special characteristics, decrease the water loss of the wound layer, prevent microbial contamination, preserve moisture and promote the healing process.

Hydrogels are regarded to possess greater ability for the medical purposes because of its quality of absorbing large amount of water which is a prominent component of human body. Recently, extensive research has been underway in respect of the possible applications of hydrogels in areas such as biosensors, drug delivery, hemostatic bandages and tissue engineering. Scientific and technological developments in the research and development of the hydrogel drug delivery system have been made in recent years by overcoming physiological adversities such as metabolism first pass and enhancing local action. Many methods are currently being used to treat pain, inflammation, skin diseases, skin disinfection and in the field of wound dressings as controlled release devices [3].

The curing of wounds is a dynamic mechanism that is triggered by the relaxation of tissue damage. Epithelial wound healing is based on a series of biological activities that starts with wound closure and continues into tissue repair and remodeling. While the healing cycle takes place independently and needs no support, multiple risk factors such as illness and difficulties in healing have drawn interest to facilitate the wound healing process [4]. Despite numerous progress in

pharmaceutical sciences, there are still only few treatment options that can induce the wound repair process. As a consequence the curiosity in discovering extracts of plants with wound healing potential has increased. The Piper genus belongs to the Piperaceae family and has about 700 species in the tropical and subtropical regions. Some of these plants are essential economically and others are used as spices and as medicines [5].

**MATERIALS AND METHODS:** Piperine was procured from Sigma Aldrich, Mumbai, Xanthan gum was procured from Loba Chemical Pvt.ltd, Mumbai, Acetic acid and Glutaraldehyde was procured from Paxmy, Speciality Chemicals, Chennai. All the chemicals and reagents used in the study were of analytical grade.

#### **PREPARATION OF CROSS-LINKED XANTHAN GUM HYDROGEL:**

The hydrogels were prepared by cross-linking method with the different concentrations of xanthan gum. A specific

Ingredients	F1	F2	F3	F4	F5	F6	F7
Piperine(mg)	100	100	100	100	100	100	100
Xanthan gum (g)	0.5	1.0	1.5	2.0	2.5	3.0	3.5
Acetic acid(ml)	40	40	40	40	40	40	40
Glutaraldehyde(ml)	1	1	1	1	1	1	1
Acetone(ml)	1.5	1.5	1.5	1.5	1.5	1.5	1.5

#### **CHARACTERIZATION:**

**Fourier Transfer infrared Spectrophotometer (FTIR):** IR spectral analysis of pure drug and physical mixtures of

quantity of xanthan gum was soaked in 40.0ml of 2.0% aqueous acetic acid at room temperature for 24 hours to obtain a viscous xanthan gum solution. A Few drops of 0.5% tween -80 was added to the solution for uniform dispersion and to prevent aggregation at ambient temperature during stirring. The solution was filtered with a sintered glass crucible and 1ml of 0.1% aqueous glutaraldehyde solution was added to obtain crosslink densities of 1.50 and stir continuously for 30 minutes at room temperature to give increased viscosity. This solution was there after casted into a Petri dish and dried overnight at room temperature to form the cross-linked xanthan gum hydrogels.

#### **Preparation of cross-linked piperine hydrogel:**

To load the Drug into the prepared cross-linked xanthan gum hydrogels, take about 10g of above prepared gel and add 0.1g of piperine and stir well.

drug & polymers were analyzed by ATR-FTIR spectroscopic technique to investigate the possible interactions. The spectra of physical mixtures and pure drug were taken by

scanning in the wave number range of 4000-400  $\text{cm}^{-1}$  using FTIR spectrophotometer (PerkinElmer; Model -Spectrum II, Chennai). Pike single bounce attenuated total reflectance (ATR) method was adopted for scanning. The peaks and patterns produced by the pure drug were compared with the peaks and patterns of the combination of drug and polymers.

**Measurement of Homogeneity:** The formulated hydrogels were visually examined for the appearance and the existence of any aggregates after they had been set in to the final containers.

**Measurement of pH:** The pH of various gel formulations was determined by using digital pH meter. 1 gram of gel was dissolved in 100 ml distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate and average values were calculated.

**Spreadability:** For the evaluation of spreadability, two glass slides were taken and the prepared gel was placed between the two glass slides pressed uniformly by applying 1000gm weight for about 4-6 minutes. And weight of about 50g is added to that. The value of spreadability is obtained by calculating the time taken for the two glass slides to detach, i.e. movement of upper glass slide over the lower one is determined as the spreadability. Lesser the time taken for

separation of two slides, better the spreadability

Using the formula;

$$S = ML / T$$

Where; S = Spreadability

M = Weight tied to upper slide

L = Length of the glass slide

T = Time taken to separate the slides from each other.

**Rheological Studies:** The viscosity of formulated hydrogels were determined by using Brook-Field viscometer DE-V model using spindle no 61 and spindle speed of 50 rpm at 37<sup>o</sup> C. Change in viscosity of the product is indicative of change in stability and effectiveness of product. Generally consistency of formulation depends on the ratio of solid fraction to liquid fraction which produces gel structure. The formulations were rotated at 0.3, 0.6 and 1.5 rotations per minute. At each speed, the corresponding dial reading was noted. The viscosity of the gel was obtained by multiplication of the dial reading with factor given in the Brookfield Viscometer catalogues.

**Surface Morphology by FE-SEM:** The surface morphology of the formulated hydrogel was determined for by using Field Emission Scanning Electron Microscopy (FE-SEM). A single drop of formulation was placed on a carbon film coated copper grid for analysis. Studies were performed at 80 kv using (FEI-QUATTRO, Chennai). The copper grip was fixed in to sample holder and placed

in a vacuum chamber of the field emission scanning electron microscope and observed under low vacuum and SEM images were recorded.

**Drug content:** For the amount of drug in the formulated hydrogels, 1mg of hydrogel was taken in to the 100ml of volumetric flask and diluted with the buffer solution pH 7.4 up to the mark. The absorbance of the sample was determined spectrophotometrically at 342.5 nm, against the blank solution of buffer pH 7.4

**Percentage Yield:** The practical yield of the sample is calculated by weighing the empty container in which the hydrogel is stored and again weighs the container along with the gel formulation and Subtraction of empty container with the container along with the gel. Percentage yield was calculated by the formula

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theoretical Yield}} \times 100$$

**Degree of swelling:** The formulated hydrogels were allowed to swell in the buffer solution of pH 7.4 for 24 hrs at room temperature. The weight of the hydrogels was checked at specific time intervals by using an electronic balance. The extent of equilibrium swelling of a hydrogel was reached when the weight of the swollen hydrogel was constant. All the experiments were performed in triplicate and the mean values were taken considering the

coefficient of variation within the range. The swelling ability of the hydrogels are calculated by the equation:

$$S = \frac{W_s - W_d}{W_d}$$

Where; S – Degree of Swelling  
W<sub>s</sub> - Weight of swollen hydrogel  
W<sub>d</sub> - Weight of dried hydrogel

**In-vitro Permeation study:** The in vitro release experiments were carried out by using Franz-diffusion cells apparatus from different formulations. An exact amount of formulations (1 g) was spread out on membrane positioned between the donor and receptor chambers with an available diffusion area. The receptor compartment was filled with phosphate buffer pH 6.8 and continuously stirred with a small magnetic bar at a speed of 50 rpm during the experiments to ensure homogeneity and maintained at 37.2±0.5<sup>0</sup>C. The samples were withdrawn at various time intervals and replaced with the same volume of phosphate buffer pH 7.4. Sink conditions were met in all cases. The samples were analyzed spectrophotometrically at 342.5nm.

**Accelerated Stability study:** The stability studies were done on the optimized formulation according to International Conference on Harmonization (ICH) guidelines. The formulation packed in aluminium tube was subjected to accelerated stability testing for 3 months as per ICH

norms at a temperature ( $40 \pm 2^\circ\text{C}$ ) and relative humidity  $75 \pm 5\%$ . Samples were taken at regular time intervals of 1 month for over a period of 3 months and analyzed for the change in pH, spreadability, drug content and in-vitro drug release. Any changes in evaluation parameters, if observed were noted. Tests were carried out in triplicate and mean value of the observed values was noted along with standard deviation.

## RESULTS AND DISCUSSION

**Homogeneity and Extrudability:** Visual examination is done to ensure the syneresis and colour of the prepared hydrogel. The hydrogel must be clear and transparent. The formulated hydrogels are found to have good homogeneity without any lumps and syneresis (**Table 1**).

**Measurement of pH:** The pH values of formulated hydrogel ranges from 6 - 9. Which is suitable for applying on skin and minimize irritation (**Table 2**).

**Drug Content:** The drug content was estimated for the formulated hydrogel. The drug content showed that the drug was distributed uniformly throughout the hydrogel (**Table 3**), (**Figure 2**).

### Percentage Yield and Viscosity

Percentage yield of topical gel containing piperine was in the range of 95.9-98.87%. The results are shown in **Table 4**. It

**Fourier Transfer infrared Spectrophotometer (FTIR):** The FTIR spectroscopic studies were carried out for standard piperine, xanthan gum and mixture of piperine, xanthan gum by ATR-FTIR technique. The FTIR spectrum of standard is compared with that of mixture and found that there is no interference. FTIR spectra of piperine, xanthan gum and mixture of piperine, xanthan gum are shown in **Figure 1**.

was found that the percentage yield of F4 formulation has greater % yield than other formulations. Generally consistency of formulation relies upon on the ratio of solid fraction to liquid fraction which produces gel structure.

**Spreadability:** For all the prepared formulations spreadability test is carried out. With respect to the increase in the concentration of polymer the spreadability of the hydrogel formulation decreases (**Table 5**, **Figure 3**).

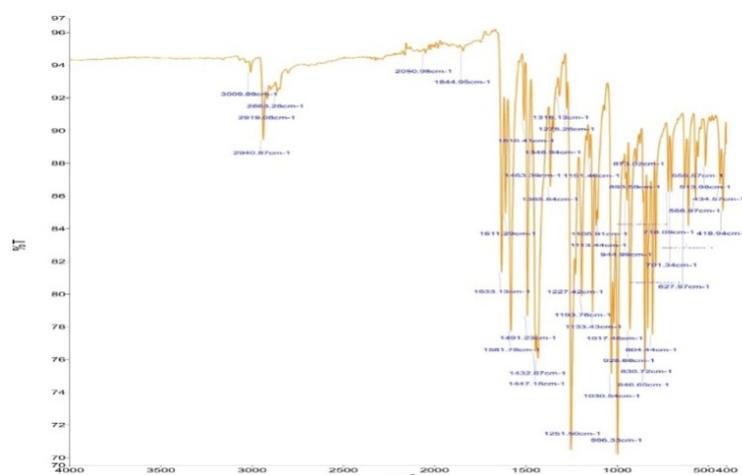
**Surface Morphology:** Field Emission Scanning electron microscope (FE-SEM) was used to study the surface morphology of cross-linked loaded polymeric hydrogel system. SEM images of piperine loaded hydrogel formulations shows coarse, rough and wavy surface (**Figure 4**).

### In-vitro Drug Release

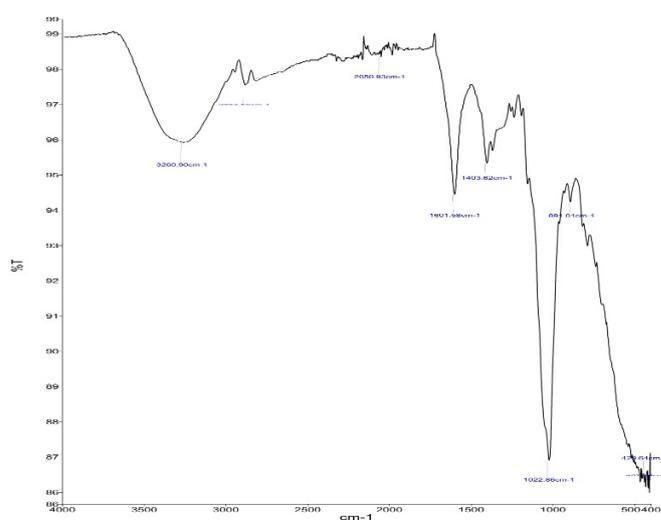
The percentage drug release of all formulations after 4.5 hours using xanthan gum was found to be 84.79 % ( F1), 89.62 % (F2), 90.54 % (F3), 92.34 % (F4), 91.79 % (F5), 90.19 % (F6) and 89.55 % (F7) respectively. It was observed that the most influenced factors in the drug release was concentration of polymer. Results of the in-vitro release studies of all formulations are

presented in **Table 7** and the graphical representation of the % drug Release data is shown in **Figure 5**.

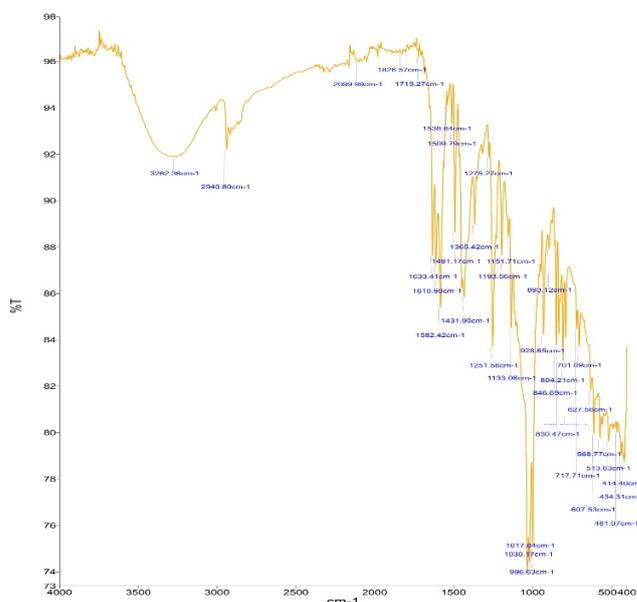
**Swellability studies:** **Table 6** shows the values of the swelling rate constant  $k$  for the hydrogels swelled in different aqueous media.



a) FTIR spectrum of Drug, Piperine.



b) FTIR spectrum of Polymer, Xanthan gum



c) FTIR spectrum of Drug + Polymer

Figure 1: FTIR Spectra of Piperine (a), Xanthan gum (b) and mixture of piperine & xanthan gum (c)

Table 1: Homogeneity and Extrudability of Hydrogel formulations

Formulation	Homogeneity	Extrudability
F1	Good	+++
F2	Very good	++
F3	Good	+++
F4	Very good	+++
F5	Very good	++
F6	Good	+++
F7	Good	++

2: pH of Hydrogel formulations

Formulation	pH
F1	6.6
F2	7.1
F3	7.5
F4	8.2
F5	7.6
F6	6.4
F7	6.0

Table 3: Drug Content of Hydrogel Formulations

Formulation	Drug content %
F1	95.32± 0.012
F2	96.32± 0.012
F3	97.43± 0.024
F4	98 ± 0.023
F5	96.32± 0.015
F6	97 ± 0.012
F7	97 ± 0.018

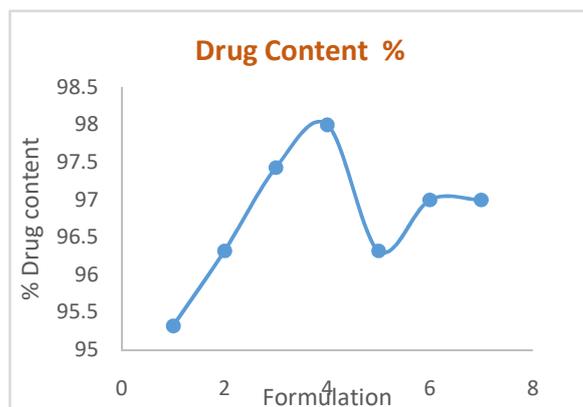


Figure 2: Drug Content ranges of Hydrogel Formulations

Table 4: Percentage Yield and Viscosity of Hydrogel formulations

Formulation code	Viscosity (centipoises)	Percentage yield %
F1	4502	96.8
F2	4321	95.9
F3	3321	97.87
F4	3741	98.87
F5	2431	98.1
F6	2323	97.39
F7	2320	96.03

Table 5: Spreadability studies of Hydrogel formulations

Formulations	Spreadability gm.cm <sup>2</sup>
F1	10.75
F2	10.93
F3	11.08
F4	11.75
F5	10.25
F6	10.64
F7	10.75

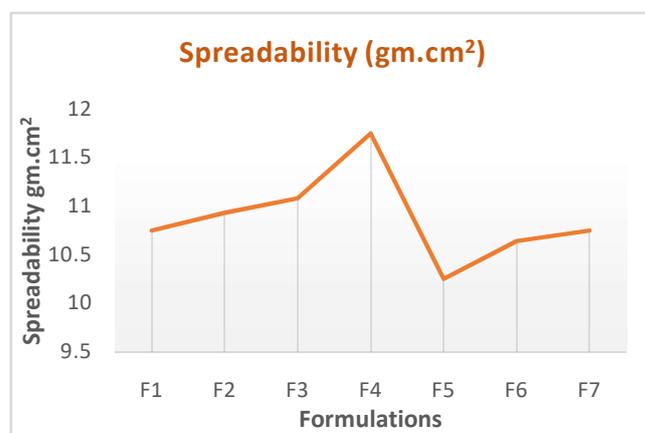


Figure 3: Spreadability studies of Hydrogel formulations

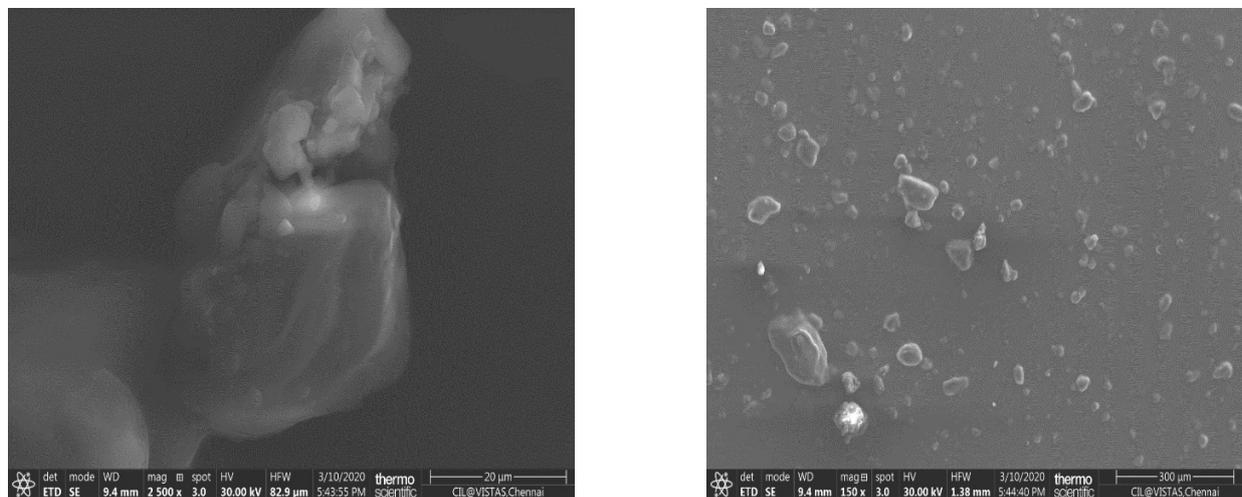


Figure 4: SEM images of Hydrogel formulations

Table 6: Values of the swelling rate constant k for the hydrogels swelled in different aqueous media

Xanthan gum concentration [wt%]	k (acid medium)	k (water)	k (basic medium)
0	0.029	0.026	0.06
5	0.037	0.046	0.09
10	0.087	0.079	0.089
15	0.124	0.097	0.134
20	0.285	0.68	0.038

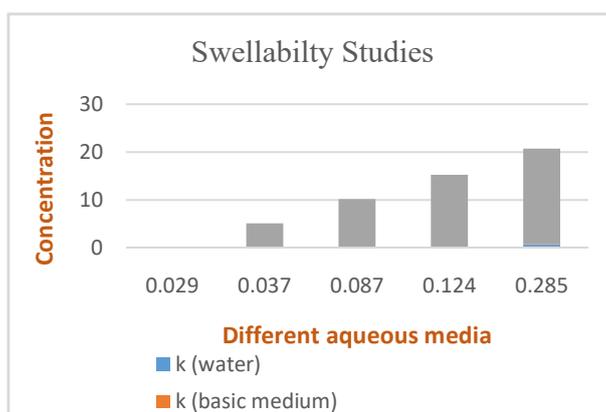


Figure 5: Swellability studies of Hydrogel formulations with different Aqueous media

Table 7: % Drug Release data of Hydrogel formulations

S. No	Time (min)	% Drug Release						
		F1	F2	F3	F4	F5	F6	F7
1	30	10.86±0.012	9.54±0.015	11.45±0.014	8.90±0.012	11.01±0.013	10.96±0.015	8.54 ±0.012
2	60	25.56±0.015	21.07±0.012	22.79±0.016	20.06±0.014	21.07±0.015	25.56±0.014	21.05 ±0.015
3	90	33.12±0.014	33.91±0.018	35.41±0.015	35.55±0.015	34.41±0.012	38.12±0.016	33.71 ±0.018
4	120	52.23±0.015	47.44±0.015	47.33±0.020	48.23±0.018	47.33±0.018	52.23±0.019	52.24 ±0.020
5	150	66.45±0.018	59.71±0.014	57.21±0.024	60.10±0.015	57.21±0.020	64.45±0.021	65.31 ±0.022
6	180	75.07±0.012	72.23±0.021	75.39±0.023	71.86±0.024	70.39±0.016	75.07±0.018	71.39 ±0.026
7	210	75.34±0.020	85.86±0.019	82.05±0.026	83.24±0.018	83.50±0.024	85.34±0.022	80.12 ±0.019
8	240	80.33±0.018	87.56±0.024	88.80±0.020	91.49±0.020	89.80±0.018	88.33±0.020	85.16 ±0.021
9	270	84.79±0.024	89.62±0.023	90.54±0.024	92.34±0.023	91.79±0.021	90.19±0.019	89.55 ±0.018

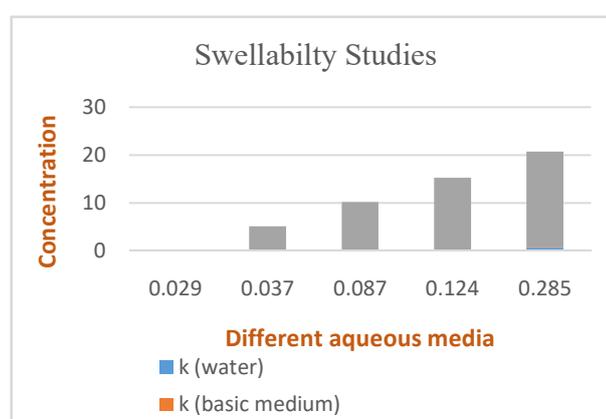


Figure 5: Graphical Representation of the % Drug Release of Hydrogel formulations

## CONCLUSION

There have been considerable improvements in the characteristics of hydrogels used in the availability of medications. More changes are therefore to be made in order to enhance hydrogels' applicability. The appropriate hydrogel material, cross-linking method and processing techniques can be chosen to achieve desirable hydrogel characteristics for a selected application.

Cross-linked polymeric hydrogel membranes loaded with the piperine were synthesized successfully with an optimized process of cross linking which would act as new healing system

for the wound healing burns with controlled release manner at wound site by preventing infection and increased healing efficiency through a contact.

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