Journal of Medical Pharmaceutical And Allied Sciences
RESEARCH ARTICLE

www.jmpas.com
ISSN 2320-7418

DESIGN AND CHARACTERIZATION OF HYDROGEL FORMULATIONS CONTAINING ALOE VERA AND NEEM SEED OIL

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Keywords
Transdermal, Hydrogel, Aloe Vera Extract, Neem Seed Oil, Carbopol, Anti-Bacterial Activity

Received
20/07/2019
Reviewed
22/07/2019
Revised/ Accepted
25/07/2019

ABSTRACT

Main aim and objective of the present study is to reduce the resistance caused by the oral The antibiotics which are used in the treatment of the dermatological infections by formulating the transdermal hydrogels by using Aloe Vera extract and Neem Seed oil as active ingredients, Carbopol 934 is used as polymer, Triethanolamine is used as pH and consistency modifier, Methyl Paraben and Propyl Paraben is used as Preservatives and Propylene Glycol is used as Solubility enhancer and as well as Moisturizer. The formulated hydrogel was evaluated for the Viscosity, Spreadability, Drug content and Strength of the Hydrogel and as well as it is evaluated for the Antibacterial activity by using Bacillus Subtilis.

INTRODUCTION

Hydrogels are networks having hydrophilic properties. While Hydrogels are generally prepared based on hydrophilic monomers, hydrophobic monomers are sometimes used in hydrogel preparation to regulate the properties for specific applications. Hydrogels are hydrophilic polymers and have three-dimensional network structures. These hydrogels are soluble in water and exhibit swelling/shrinking depending on various external stimuli such as temperature, pH, electric field, magnetic field, light, etc. Therefore, they have been termed as stimuli responsive hydrogels. In this work we have applied the both the methods for synthesis of novel Hydrogel membranes.

Advantages:

- Biocompatible, Biodegradable and that is why they can be injected
- Improved Drug utilization
- Drug adopts to suit circadian rhythms of body functions or diseases Lower daily cost to patient due to fewer dosage units are required by the patient in therapy
- Drug targeting to specific site like colon
- Decreased Dose of administration

Skin Anatomy: Skin comprises of three tissue layers: the upper most being the stratified, avascular, cellular epidemic which

is the outer most and nonviable layer of the skin, it acts as protective barrier the body and highly difficult to transverse.

Drug and Excipient Profile
Aloe Vera and Neem Seeds:

Aloe Vera leaves and Neem Seeds are collected from the local area of Kesanupalli (village), Narasaraopet (Mandal), Guntur(district), Andhara Pradesh, India (February 2019) and authenticated by the Pharmacognosy unit MAM College of Pharmacy.

MATERIALS AND METHODOLOGY

Sr	Name of the Ingredient	Category	Image of the Ingredient
1	Azadirachta indica	Antibiotic, Antifungal and Antiseptic	
2	Aloe Vera	Moisturizing agent, Delivers smoothening property to the skin	
3	Rose Water	Masks the unpleasant odor of the Neem seed oil and also quite effective against acne	

Table 1: List of Chemicals

Name	Use/Category
Carbopol 934	Jellying agent
Methyl Paraben	Preservative
Propyl Paraben	Preservative
Propylene Glycol	Solubility enhancer
Triethanolamine	pH modifier

Table 2: Composition of Extract

Name of the Ingredient	Composition
Extract of Neem Seeds	1gm
Extract of Aloe Vera	2gm

METHOD OF PREPARATION OF HYDROGEL

Containing Aloe Vera and Neem Seed oil:

1g of Carbopol 934 was dispersed in 50 ml of distilled water kept the beaker a side to swell the Carbopol 934 to form gel. Take 5ml of distilled water and required quantity of methyl Paraben and propyl Paraben were dissolved by heating on water bath solution was cooled and propylene glycol and sodium lauryl sulphate added. Further required quantity of extract was mixed to the above mixture and add this solution into the Carbopol 934 gel with continuous stirring and add Triethanolamine was added drop wise to the formulation for adjustment of required skin pH and to obtain the gel at required consistency.

EVALUATION TESTS FOR HYDROGEL FORMULATIONS CONTAINING ALOE VERA AND NEEM SEED OIL:

PHYSICAL EVALUATION

Physical parameters such as color, odor and consistency were checked manually.

SOLUBILITY

Freely soluble in aqueous media

WASH ABILITY

The product was applied on hand was observed under running water.

pH

pH of 1% aqueous solution of the formulation was measured by using a calibrated digital pH meter at constant temperature.

SPREADABILITY

Spreadability was determined by the apparatus which consists of a wooden block, which was

provided by a pulley at one end. By this method Spreadability was measured on the basis of slip and drag characteristics of gels. An excess of gel (about 2 g) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. A 1 kg weighted was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 80 g. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm be noted. A shorter interval indicates better Spreadability. Spreadability was calculated using the following formula:

$$S = M \times L / T$$

Where, S = Spreadability,
M = Weight in the pan (tied to the upper slide)
L = Length moved by the glass slide and
T = Time (in sec.) taken to separate the slide completely each other.

EXTRUDABILITY

The gel formulation was filled in standard capped collapsible aluminum tubes and sealed by crimping to the end. The weight of tubes was recorded and the tubes were placed between two glass slides and were clamped. 500gm was placed over the slides and then the cap was removed. The amount of extruded gel was collected and weighed. The percent of extruded gel calculated as:

1. When it is greater than 90% then Extrudability is excellent.
2. When it is greater than 80% then Extrudability is good.
3. When it is 70% then Extrudability is fair.

IRRITANCY TEST

The cream was applied on left hand dorsal side surface of 1sq.cm and observed in equal intervals up to 24hrs for irritancy, redness and edema.

FOAM ABILITY

Small amount of gel was taken in a beaker containing water. Initial volume was noted, breaker was shaken for 10times and the final volume was noted. Foam ability was also analyzed by applying on skin with water.

STABILITY STUDIES

The stability studies for optimized formulation were conducted in the accelerated conditions as per guidelines of International Conference on Harmonization (ICH). Well closed container was used for the storage of optimized gel formulation. The gel formulations were stored at 40˚C and 75% relative humidity for 30 days. Samples were drawn at aforethought time interval of 30 days. The gel formulation was evaluated for their physical properties including appearance, color, and presence of clogs, consistency and phase separation.

GRITTINESS

The product was checked for the presence of any gritty particles by applying it on the skin

SWELLING STUDIES

The pH dependent swelling property of the hydrogels was studied in both 1.7pH of 0.1N HCl acidic medium and 7pH of distilled water basic medium. 20mg of hydrogel without drug was placed in 30ml solution of HCl and water for time interval of 10mins till 1hour. At every 10minute interval, the hydrogels were removed and excess surface liquid was removed by blotting paper and their weights were recorded. Another study was carried out by taking 39mg of hydrogel without drug and the same process was repeated with 1 hour of time interval for 5hours.The percentage swelling (S) was determined by the following equation,

$$q_{eq} = (W_h/W_d)$$

Where W_h-weight of swollen Hydrogel at the Time t,W_d-initial weight of dried Hydrogel

DIFFUSION STUDIES

The required length of egg membrane was cut and tied or glued to the bottom (grounded) layer of diffusion cell with a thread to form an inner compartment. 10 ml of 0.1N HCL were added to the inner compartment and placed in a beaker containing 100 ml of 0.1 N HCL, which acts as an outer compartment. Care was taken to make sure that the level of media in both compartments is equal. A magnetic bead was added to outer compartment to stir the contents during the studies. The assembly was placed in a magnetic stirrer and temperature was maintained at 37degree Celsius. the weighed amount of the pure drug is added to inner compartment of the diffusion cells and studies where performed for duration of 30 min. at predetermined time intervals (5 min, 10 min , 15 min , 20 min , 25 min , 30 min.) , samples were withdrawn and the same volume of media was replenished to maintain the sink volume. The solutions were suitably diluted and the absorbance was measured spectrophotometrically at 250 to 800 nm.

Plot the graph between time on x-axis vs % of drug release on y-axis from the table and slope was calculated as following:

Slope = y2-y1/x2-x1

Amount of drug release = concentration *volume of dissolution medium/1000

% drug release =amount of drug release *dilution factor/drug dose

ANTI-BACTERIAL ACTIVITY MEASUREMENT

The principle of cup and plate method is based on diffusion of an antibiotic solution from the cavity through the solidified agar of petri plate used for the study. The growth of microbes is not found in circular area round the cavity called "zone containing solution of antibacterial". In this prepared hydrogel, Bacillus Subtilis is used as the test microorganism and medium E is used as the medium for growth of test culture.

METHOD FOR ACTIVITY

1. Required amount of hydrogel was dissolved in 100 ml of sterile water and dilutions are made with this solution
2. pure drugs were taken as standard
3. sterile the assay medium by autoclave and prepare petri plates and laminar air flow
4. spread the test microorganism on the surface of petri plates by spread plate technique
5. By using flame sterilized cork borer, prepared 5-6 cups in each plate keeping adequate distance from each other.
6. Standard and diluted solutions of samples depending upon the size are added in each labelled cavity of plates.
7. Transfer all the plates in which for proper diffusion at anti-bacterial at 4C for 1-2 hrs.
8. Incubate all the plates in incubator and note down the results and draw a graph.

RESULTS AND DISCUSSION

From the results it is concluded that all the hydrogel formulations showed good appearance and homogeneity. The physical appearance of hydrogel formulations was white in nature. The pH of the hydrogel formulations was in the range of 6.8 – 7, which lies in the normal pH range of the skin and with time no skin irritation was observed. There was no significant change in the pH values with time (varied from 0.05 to 0.20).

The values of Spreadability indicated that the hydrogels were easily spreadable by small amount of shear. Spreading diameter after one minute (mm) of prepared gel was

between 38-55mm which indicates good Spreadability of herbal hydrogels. The results of skin irritancy studies indicated that the prepared hydrogels were free from dermatological reaction. The hydrogels were evaluated for anti- bacterial activity by using cup plate method.

Results of Antibacterial Activity of the Hydrogel Containing Aloe Vera and Neem Seed oil:

The release of the active ingredient from the gel was varied according to the concentration of the polymer. The progressive increase in the amount of drug diffusion through the semipermeable membrane from formulation F6 and F7 attributed to gradual decrease in the concentration of the polymer. It has been concluded that, if we increase the polymer concentration, the diffusion of the drug through the skin also decreases. The amount of the drug diffused from the formulation F1 and F4 were 98.55 and 98.99 respectively in 30min which were the higher among the all Hydrogel formulations. The order of drug diffused from various formulations was found to decreasing in the following order.

F4>F1>F8>F2>F3>F5>F7>F6

By the statistical analysis showed that the inhibition by formulation containing extracts were significantly differing from control group at all the concentration tested. the results showed that the anti-bacterial effect of the formulation containing 4% aloe Vera and azadirachta indica hydrogel was better than the effect of other hydrogel formulation. By the results, it is found that all hydrogel formulations are having the desired antibacterial activity at the concentrations of 0.669 to 0.890. From the all formulations f1 and f4 are having the maximum antibacterial activity. The results obtained from the remaining formulations are not having that much of variations in antibacterial activity and are within the limits of its activities. the antibacterial activity of extract shows the increased the linearity with the increase of the concentration.

CONCLUSION

Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Herbal formulation shares growing demand in the world market. it is very good attempt to establish the herbal hydrogel containing aqueous extracts of Neem oil , aloe vera, rosewater. The plants have been reported in literature having antimicrobial, antifungal, antiseptic, moisturizing, soothing effects and quite effective aganist to acne. Formulation are prepared by using varied concentration of extract prepared formulation (f1 to f8) where evaluated for various parameters like colour, appearance, consistency, washability, pH and spreadability, extrudability, skin irritation.

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EXPERIMENTAL TABLE AND FIGURE

Table3: Formulae for the Formulation of Hydrogels Containing Aloe Vera & Neem Seed oil extract:

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Aloe Vera gel and Neem oil	4g	4g	4g	4g	4g	4g	4g	4g
Carbopol 934	1g	1g	2g	2g	3g	3g	1.5g	2.5g
Propylene glycol	5ml	5ml	5ml	5ml	5ml	5ml	5ml	5ml
Methyl paraben	0.2g	0.2g	0.2g	0.2g	0.2g	0.2g	0.2g	0.2g
Propyl paraben	0.1g	0.1g	0.1g	0.1g	0.1g	0.1g	0.1g	0.1g
Triethanolamine	2ml	1ml	2ml	1ml	2ml	1ml	2ml	1ml
Rose water	25ml	25ml	25ml	25ml	25ml	25ml	25ml	25ml
Distilled water	q.s to 50ml	q.s to 50ml	q.s to 50ml	q.s to 50ml	q.s to 50ml	q.s to 50ml	q.s to 50ml	q.s to 50ml

Table 4: Results of Evaluation Parameters

Parameter	F1	F2	F3	F4	F5	F6	F7	F8
Color	White	White	White	White	White	White	White	White
Odor	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant
Consistency	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Solubility	Freely soluble in water	Freely soluble in water	Freely soluble in water	Freely soluble in water	Freely soluble in water	Freely soluble in water	Freely soluble in water	Freely soluble in water
Wash ability	Washable	Washable	Washable	Washable	Washable	Washable	Washable	Washable
pH	6.8	6.7	6.9	6.8	7.0	6.7	6.9	6.8
Spreadability	Good	Good	Good	Good	Good	Good	Good	Good
Extrudability	Good	Good	Good	Good	Good	Good	Good	Good
Irritancy	No	No	No	No	No	No	No	No
Foam ability	No	No	No	No	No	No	No	No
Grittiness	No	No	No	No	No	No	No	No

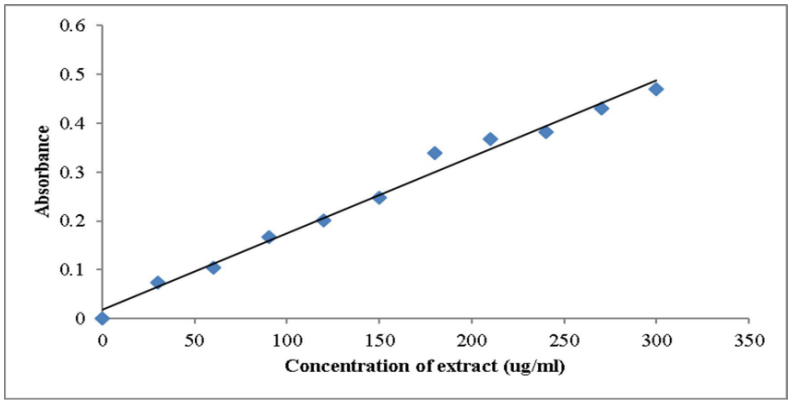

Figure 1: Calibration Curve of Extract Containing Aloe Vera and Neem oil in 0.1 N HCl

Table 5: % Dissolution Drug Release

S.NO	TIME (MIN)	ABSORBANCE	CONCENTRATION (mg/ml)	AMOUNT OF DRUG RELEASE	% OF DRUG RELEASE
1.	5	0.270	0.500	450.45	90.23
2.	10	0.315	0.570	461.49	92.61
3.	15	0.325	0.651	468.33	93.63
4.	20	0.435	0.759	472.35	94.66
5.	25	0.476	0.799	483.50	97.63
6.	30	O.530	0.863	491.18	98.55

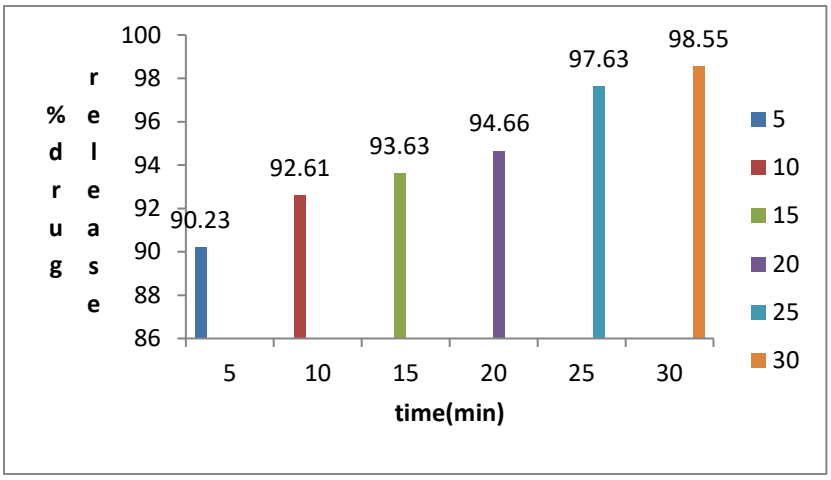

Figure 2: Diffusion studies of Formulation-1

Table 6: Diffusion Studies of Hydrogel Formulation Containing Aloe Vera & Neem Seed oil (Formula-2)

TIME IN (MIN)	ABSORBANCE	CONCENTRATION	AMOUNT OF DRUG RELEASE	% DRUG RELEASE
5	0.268	0.480	415.23	83.9
10	0.310	0.507	432.91	89.67
15	0.320	0.621	453.72	91.76
20	0.415	0.695	463.10	92.99
25	0.435	0.730	480.25	95.68
30	0.470	0.790	485.75	97.85

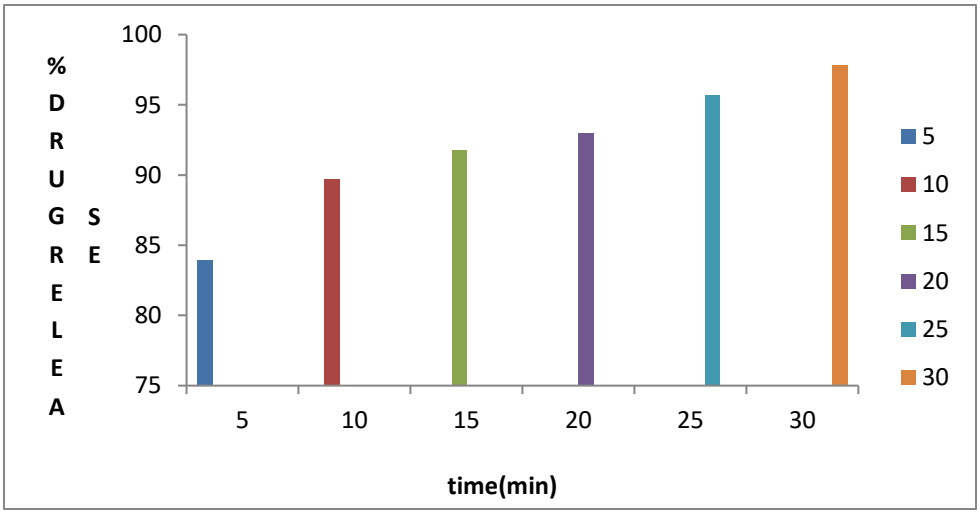

Figure 3: Diffusion Studies of Formulation-2

Table 7: Diffusion Studies of Hydrogel Formulation Containing Aloe Vera & Neem Seed oil (Formula-3)

TIME IN (MIN)	ABSORBANCE	CONCENTRATION	AMOUNT OF DRUG RELEASE	% OF DRUG RELEASE
5	0.243	0.634	423.98	86.56
10	0.257	0.658	439.86	88.56
15	0.264	0.689	453.78	90.35
20	0.296	0.720	467.39	93.87
25	0.340	0.748	479.92	95.64
30	0.398	0.760	480.02	96.78

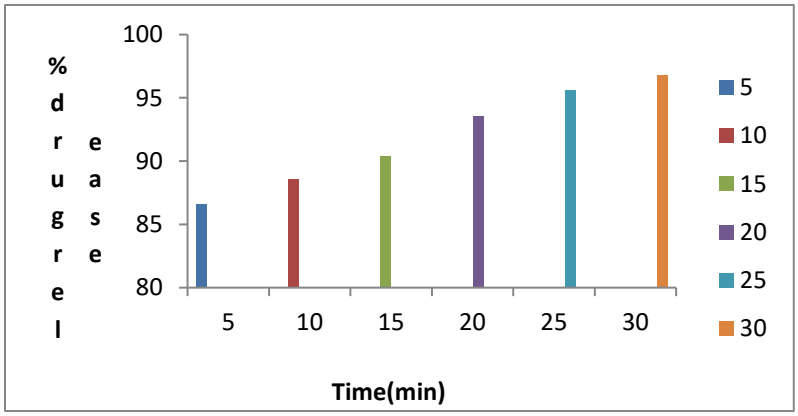

Figure 4: Diffusion Studies of formulation-3

Table 8: Diffusion Studies of Hydrogel Formulation Containing Aloe Vera & Neem Seed oil (Formula-4)

TIME IN (MIN)	ABSORBANCE	CONCENTRATION	AMOUNT OF DRUG RELEASE	% DRUG RELEASE
5	0.254	0.696	0.434	93.61
10	0.278	0.712	0.456	94.23
15	0.298	0.730	0.473	94.98
20	0.345	0.753	0.481	95.86
25	0.370	0.770	0.489	97.54
30	0.394	0.790	0.495	98.99

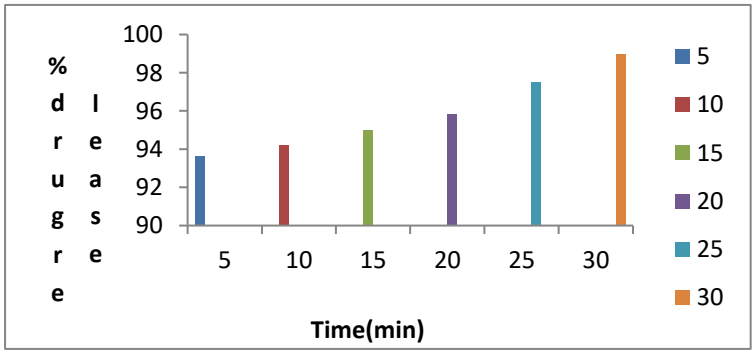

Figure 5: Diffusion Studies of Formulation-4

Table 9: Diffusion Studies of Hydrogel Formulation Containing Aloe Vera & Neem Seed oil (Firmula-5)

TIME IN (MIN)	ABSORBANCE	CONCENTRATION	AMOUNT OF DRUG RELEASE	% DRUG RELEASE
5	0.231	0.624	0.409	92.21
10	0.247	0.636	0.421	93.32
15	0.269	0.643	0.429	93.47
20	0.289	0.674	0.435	94.35
25	0.299	0.680	0.449	95.12
30	0.320	0.702	0.458	96.21

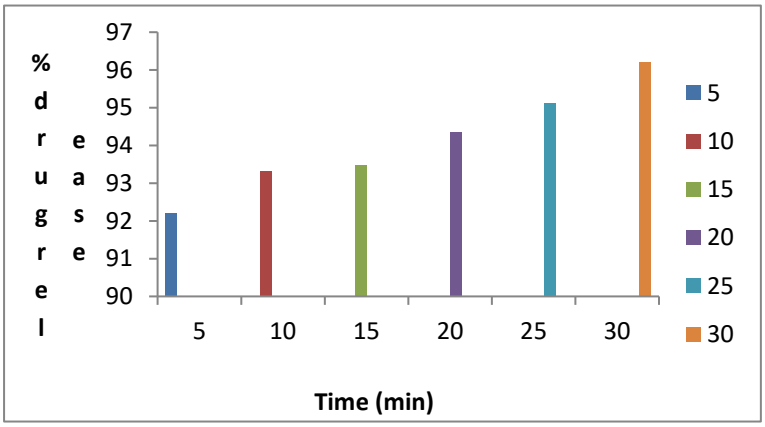

Figure 6: Diffusion Studies of Formulation-5

Table 10: Diffusion Studies of Hydrogel Formulation with Aloe Vera & Neem Seed oil (Formula-6)

TIME IN (MIN)	ABSORBANCE	CONCENTRATION	AMOUNT OF DRUG RELEASE	% DRUG RELEASE
5	0.224	0.623	0.409	91.22
10	0.243	0.632	0.421	92.00
15	0.265	0.640	0.429	92.85
20	0.289	0.654	0.435	93.43
25	0.297	0.660	0.449	94.12
30	0.332	0.669	0.458	94.65

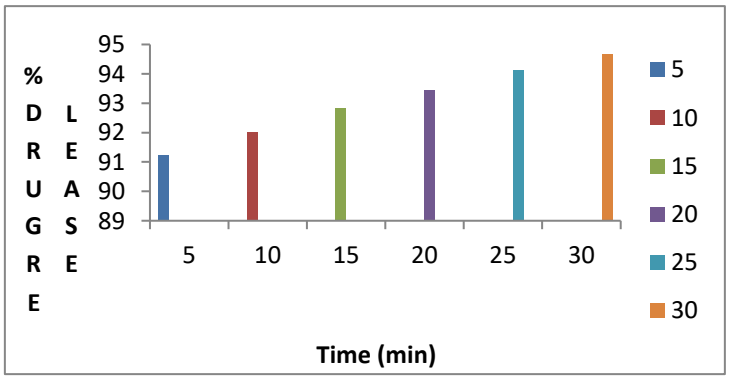

Figure 7: Diffusion Studies of Formulation-6

Table 11: Diffusion Studies of Hydrogel Formulation Containing Aloe Vera & Neem Seed oil (Formula-7)

TIME IN (MIN)	ABSORBANCE	CONCENTRATION	AMOUNT OF DRUG RELEASE	% DRUG RELEASE
5	0.237	0.632	0.421	92.12
10	0.243	0.641	0.429	92.89
15	0.249	0.654	0.435	93.34
20	0.258	0.659	0.446	93.99
25	0.263	0.663	0.452	94.59
30	0.287	0.670	0.459	95.00

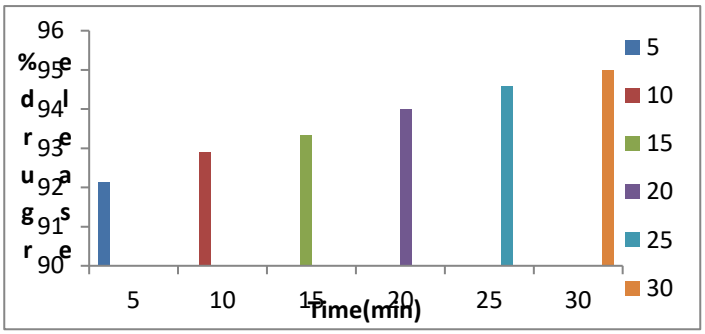

Figure 8: Diffusion Studies of Formulation-7

Table 12: Diffusion Studies of Hydrogel Formulation Containing Aloe Vera and Neem Seed oil (Formula-8)

TIME IN (MIN)	ABSORBANCE	CONCENTRATION	AMOUNT OF DRUG RELEASE	%DRUG RELEASE
5	0.235	0.630	0.423	93.01
10	0.241	0.639	0.431	93.99
15	0.257	0.648	0.439	94.34
20	0.262	0.653	0.443	95.23
25	0.270	0.660	0.454	96.57
30	0.279	0.669	0.462	97.99

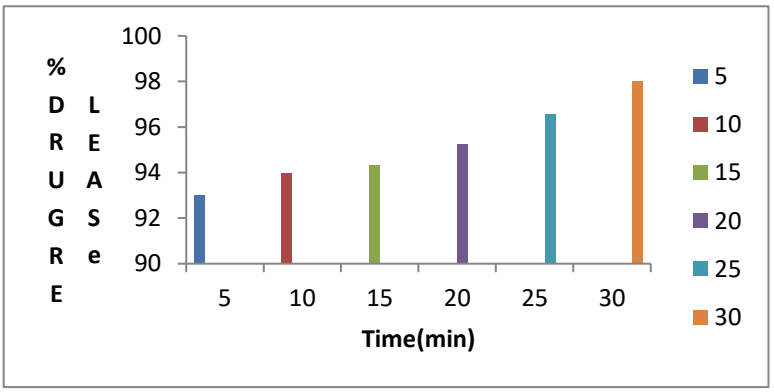

Figure 9: Diffusion Studies (formulation-8)

Table 13: Antibacterial Studies of Hydrogel Formulations Containing Aloe Vera and Neem seed oil:

BATCH	CULTURE	ZONE OF INHIBITION
F1	Bacillus subtilus	19.5
F2	Bacillus subtilus	10.6
F3	Bacillus subtilus	11.5
F4	Bacillus subtilus	19.07
F5	Bacillus subtilus	14.10
F6	Bacillus subtilus	15.83
F7	Bacillus subtilus	16.69
F8	Bacillus subtilus	18.72
STANDARD	Bacillus subtilus	20.5

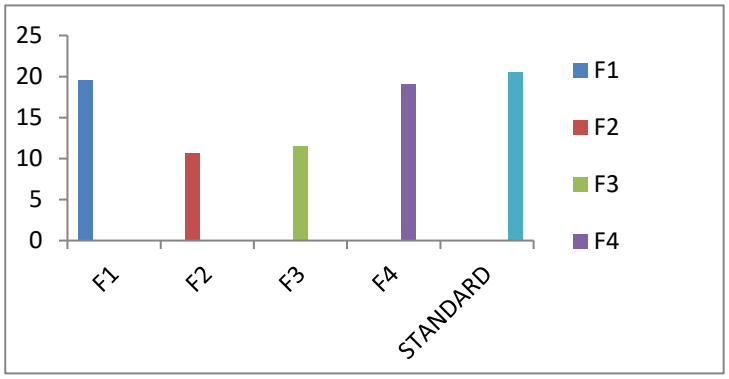

Figure10: Antibacterial effect-1

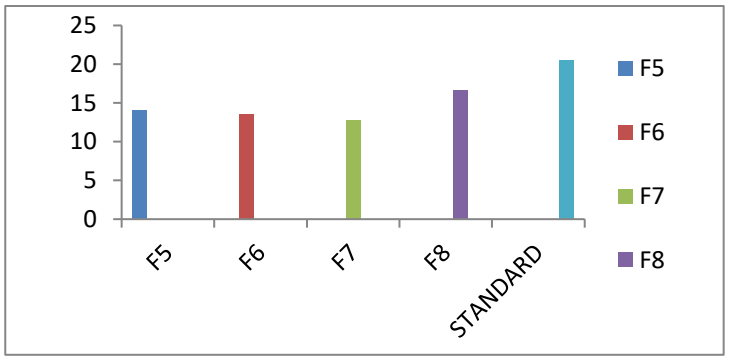

Figure 11: Antimicrobial effect-2