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"Formulation and Evaluation of Clindamycin phosphate Nanoparticles foam as a carrier drug delivery system for the treatment of acne vulgaris".

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ABSTRACT: The present research is based on developing topical nanoparticles-foam formulation of clindamycin phosphate for the treatment of acne vulgaris. Foam is act as a best carrier system for the topical nanoparticles. Nanoparticles system have capacity to improve the topical drug delivery of drug because of their increasing drug loading potential, but the commercially use of nanoparticles in topical product is limited because the penetrate intact skin is contradictory and also their ability to release active agent in traditional semisolid vehicle is poor. To solve this problem foam formulation is developed. This is targeted or site specific formulation for the treatment of acne vulgaris. This foam formulation have the potential to break down the nanoparticles loaded within them improve the drug release from nanoparticles. Nanoparticles prepared by ionic elation nano-precipitation method by using the sodium alginate and guar gum biodegradable natural polymers. The DSC study of polymer with drug shows the endothermic peak at 95.866°C and the SEM study of batch shows the cubic shape nanoparticles and somewhat irregular shape and the drug being entrapped in the polymeric material. The entrapment efficiency of A4 batch is 75% greater than other all batches, on the basis of particle size ,zeta potential A4 batch shows the greater entrapment efficiency good in particle size and zeta potential. The cetyl alcohol and stearyl alcohol ration up to (60-40) or (80-20) help to maintain the foam characteristics ,make the foam quick breaking as well temperature sensitive.The HET-CAM test established non-irritancy of CLP nanoparticles loaded foam formulation.

KEYWORLDS- Foam, Acne Vulgaris, Clindamycin phosphate, nanoparticles, polymer.

INTRODUCTION[1,2] : The term acne derived from Greek word- acne which means – prime of life. It manifest at any time during life but it most commonly presents between ages of 12-14, which estimate 85% of affected population. Acne occurs most commonly during adolescence, affecting an estimated 80-90% of teenagers in the Western world. Lower rates are reported in some rural societies .In 2010 acne was estimated to affect 650 million people globally making it the 8th most common disease worldwide .The people may also be affected before and after puberty through it becomes less common in adulthood than in adolescence, nearly half of people in their twenties continue to have acne. About 4% continue to have difficulties into their forties. Several topical and oral anti-acne agents (e.g. Retinoid) in combination with other treatments (e.g. laser therapy) are generally used to treat inflammatory acne. Physicians recommend combination therapies (topical retinoid with antibiotics) to treat severe

lesions forming in the inflammatory acne. Due to side effects of oral retinoid, physicians are not in preference for its use as a single treatment in various forms of acne. If not treated it acne leads to scar formation , currently many drugs belonging to antibiotics, non-steroidal, anti-inflammatory and antioxidant being used for its treatment. Major modalities of acne treatment includes physical means use of drug.Clindamycin phosphate is the salt phosphate salt of clindamycin which is a semi-synthetic chlorinated broad spectrum antibiotic produced by chemical modification in lincomycin . The topical use of clindamycin phosphate is it stop the growth of certain bacteria at that causes the acne. Decreases the numbers of acne lesions. Drug is used to treat infection of susceptible bacteria; it can be used topically, orally or intravenously.Clindamycin phosphate is used to treat the acne

Nanoparticle[3,4] for dermatological application or polymer based nanoparticle has developed. These have in one way or the other, addressed the shortcoming of the traditional TDDS such as ointments, gels etc. Different carrier systems have been proposed in an attempt to favour the transport of drugs through the skin, enabling drug retention and in some cases allowing a controlled release Skin penetration is essential to a number of current concerns, e.g. contamination by microorganisms and chemicals, drug delivery to skin, and skin care and protection.The most used and investigatedNano carrier for dermal drug delivery in the pharmaceutical field include liposomes,transferosomes, ethosomes,niosomes and nanoparticles.

Foam[5] for topical application introduced the new vehicle to deliver drug for dermal application. Foam have more advantages than conventional vehicle. Foam absorbed more rapidly to the skin without leaving any oily ,greasy residue .there are lots of application than the ointment, gel, lotion and cream. Foam spread more easily on large areas and required less mechanical force compare to other traditional vehicle. The most important advantage of is that Foam can used to the sensitive or highly inflamed skin it may cause adverse effect or painful or cause inflammation.

MATERIAL AND METHOD:

Clindamycin phosphate, dehydrated alcohol, cetyl alcohol, stearyl alcohol NF, polysorbate 80, propylene glycol, sup potassium hydroxide, usp, Guar gum, sodium alginate,sodium bicarbonate, calcium chloride, distilled water.

Preparation of nanoparticles[6,7,8,9]: Clindamycin phosphate nanoparticles was prepared by ionotropic nano precipitation method. Nanoparticles are prepared by the dissolving sodium bicarbonate and guar gum in distilled water. And the drug was dispersed in to polymeric material. In other beaker sodium bicarbonate and calcium carbonate was dissolved in distilled water. In the drug polymeric solution was fabricated with the drop wise addition of the polymeric solution to the calcium chloride solution under magnetic stirring at room temp. The nanoparticles were separated bycentrifugation process. Nanosuspension was freeze dried and stored.

Preparation of foam drug delivery system[8]:

Method[10]: Foam is prepared by the whipping method. This method is a standard method of gas introduction to a liquid. The volume of the air incorporated into the foam usually increases with an increase of beating intensity, whereas beating of a high viscous liquids leads to the generation of unstable foams. Every air bubble undergoes severe mechanical stresses through whipping; therefore, a more rapid coalescence happens during foam generation compared to standing foam. Final foam volume is a result of dynamic equilibrium between mechanical air bubble formation and bubble destruction. The mechanical stress also leads to the destruction of bigger air bubbles into smaller ones.

FTIR analysis:Sodium alginate and guar gum nanoparticles were conjugated with Clindamycin phosphate. Due to this conjugation there may be chances ofadsorption of some functional groups to the newly formed conjugated nanoparticles. Hence, FTIR analysis was done to study the chemical properties of nanoparticles conjugated clindamycinphosphate and after knowing the functional groups its bonding nature with nanoparticles was also characterized.

Characterization of nanoparticles:

The IR spectrum of nanoparticles was obtained using a Fourier transform spectrometer (spectrum GX-1, Perkin Elmer's USA) particle size and zeta potential of nanoparticles were measured by photon correlation spectroscopy and laser Doppler anemometry (Nano- zest& MPT-2, Malvern, UK) respectively.

Determination of entrapment efficiency and loading capacity: The entrapment efficiency was calculated by using 100 mg of nanoparticles dissolved in 20 mL of dichloromethane and the solution was centrifuged at 12000 rpm. The supernated fluid was collected and passed through membrane filter. The quantity of drug in the solution was measured by ultra violet spectroscopy at 195 nm.

Drug entrapment (%) = Quantity of drug in nanoparticle/Mass of drug in the formulation×100

Differential scanning colorimetry (DSC) of nanoparticles: The physical state of Clindamycin phosphate entrapped in the nanoparticles as well as the polymers and the blank nanoparticles of sodium alginate and guar gum were characterized by differentials canning calorimetric thermo gram analysis ,The samples (~12 mg) were weighed and sealed in Aluminium pans and heated under nitrogen by heating rate of 100 C/min, the heat flow being recorded from 300C to 2000C. Indium was used as standard reference material to calibrate the temperature and energy scales of the DSC instruments. After getting data through Microsoft excel we got the DSC thermograph.

Scanning electron microscopy (SEM):

Electron micrograph of clindamycin phosphate nanoparticles was obtained using a scanning electron microscope (JEOL JSM-5200) operating between 5 and 24kV. Thespecimens were mounted on a metal stub (with double side adhesive tape) and coated under vacuum with gold in an argon atmosphere prior to observation.

Characterization of clindamycin phosphate loaded nanoparticles foam drug delivery system:

Evaluation of foam the prepared foam formulation was inspected visually for their colour, appearance and consistency.

PH determination:1% foam formulation was prepared and stored for 2 hr. and pH was determined using a digital pH meter. The pH of each foam formulation was done in the triplicate, average value and ± standard deviation was calculated.

Viscosity[13]: The viscosity of the formulation batches was determined using a Brookfield viscometer with spindle no 6 and 74rpm. Viscosity in centipoises (cp) was calculated.

Determination of foam stability and foamability[14]:

Foam stability is usually reflected by the initial foam volume and subsequent, measurements of the volume as a foam ages.A cylinder test was carried out to determine the following parameters, foam expansion, foam liquid stability , and foam volume stability ,Foam gas fraction was determined as the difference between the initial foam volume and volume of the non expanded formulation fifty ml of formulation was pour for 20min by wrist action shaker .The total volume of the foam immediately after shaking and at 1 min interval for 4 min recorded.The higher the FE the more formable is the formulation. The lower the FLS and the higher the FVS the more stable is the produced foam.The foam was discharged into a glass cylinder. The initial volume of foam, the volume of the aged foam and the volume of drained liquid after a defined time period was measured. The separation of the liquid due to liquid drainage was observed after 15 min.

Drug content: The quantity of formulation equivalent to 10 mg of was dissolved using 100ml Methanol. From this solution, 1ml sample were withdrawn and diluted to 10 ml with Methanol. This sample were analyzed spectrophotometrically at a wavelength of 210nm and concentration of clindamycin phosphate in each sample was estimated from previously prepared standard curve.

Diffusion (*in-vitro*) through biological membrane: Release of clindamycin phosphate *(in-vitro*) from the formulation was estimated using Franz diffusion cell using synthetic membrane(porosity -0. 2 micro meter). The membrane was soaked on to phosphate buffer (ph.7.4): Methanol (6:4) for 9-12hr. It was placed over the rim of receptor compartment of the diffusion cell which was filled with blends of phosphate buffer solution (ph 7.4) methanol (6:4). The quantity of formulation equivalent to 10 mg of clindamycin phosphate was spread uniformly on the membrane. This whole assembly was kept on a magnetic stirrer and the solution on the receptor side was stirred continuously using a magnetic bead, temperature of the medium was maintained at 37 +- 0.5 .A similar set was run simultaneously without application of clindamycin phosphate as a control. Sample (2ml) were withdrawn at suitable

time interval and replaced with equal amount of fresh medium. Sample was analyzed spectrophotometrically at 210 nm and the cumulative% drug diffused was calculated. The difference in absorbance between drug released from that formulation and receptor fluid in control was used for estimated of concentration of clindamycin phosphate diffused through the formulation.

***In vitro* Antibacterial study:**

Efficacy against causative bacteria (*in-vitro*): procedure:

Culture: Propionibacterium acne (MTCC-1951)

1**. Preparation of medium:**

Modified Reinforce clostridial agar / broth medium was used in the conventional manner for cultivation of bacteria. As directed by the manufacturer medium was 51.00gram of medial was suspended in 1000ml distilled water and heated to dissolve the medium completely by autoclaving at 15Ibs pressure (121c) for 15 min and used for further procedure.

2. Preparation of inoculums:

The inoculums of *propionibacterium acne* was prepared from 7 to 15 day old culture. The prepared modified reinforced clostrial agar /broth medium was sterilized by autoclaving at 121 c, 15 Ibs for 15 minutes then it was poured in the petri plates. By rotating the petri plate the medium was uniformly distributed and allowed to solidify. The mature colonies were taken by the nichrome wire loop from agar slant and transferred on agar medium by streaking the nichrome wire loop on previously prepared plates. These plates were incubated at 28 C for 5-7days for proper growth of bacterial culture.

Working procedure:

The agar well diffusion method used to evaluate efficacy against causative Bacteria of the of clindamycin phosphate. The prepared modified reinforced clostrial agar/broth medium was sterilized by autoclaving at 121 ,15Ibs for 15 minutes then it was poured in the petri plates.By rotating the petri the medium was uniformly distributed and allow solidify, From the sub cultured were taken by nichrome wire loop in the moistened in the above inoculums suspension and surface of modified reinforce clostridial agar/broth medium plates were streaked in 4 different direction (at 90 angle), so as to cover the entire surface. Using sterile cork borer the medium was bored (5mm) and the prepared test sample was taken and added in each bore. The plates were incubated at 28 c for 4-5 days. Later the diameter of zone of inhibition of bacteria were recorded. Antibacterial efficacy of selected formulation of clindamycin phosphate against *Propinibacterium acne*.

Dermal skin irritation potential (*in-vivo*) of selected clindamycin phosphate loaded nanoparticles foam :Dermal irritation study was carried out by HET-CAM (hens egg chorioallantoin membrane) test. Day 1st- Fertilized hen's eggs (three) weighing between 50 to 60 gm was selected and candled in order to discard the defective ones. These eggs were incubated in humidified incubator at a 40^{O}C for 3 days.

Day3rd- Egg albumin (3ml) was aspired off from the sharpened end of the egg using aseptic technique. For the development of CAM away from the shell, the eggs were kept in the equatorial position.

Day 5th - Of incubation, the eggs was candled and non-feasible embryos were removed. Day 10th- Formulations (0.5ml) was in stilled through window (2×2cm) on the equator. A 0.9% Nacl solution was used as a control as it is reported to be practically non-irritant. The scores were recorded according to the scoring scheme presented in the – Table No- Test-optimized formulation standard - saline and marketed formulationPositive control-0. 1M sodium lauryl sulphate (SLS)

RESULT AND DISCUSSION:

Drug –Exicipient compatibility: The COOH stretching was shows at the 2422.57. The N-H Stretching of amine group at 3302.44.The C=O stretching of carbonyl group of carboxylic acid observed at the1631.50. C-CL stretching observed at the 678.97.this shows that the drug is compatible with other excipients.

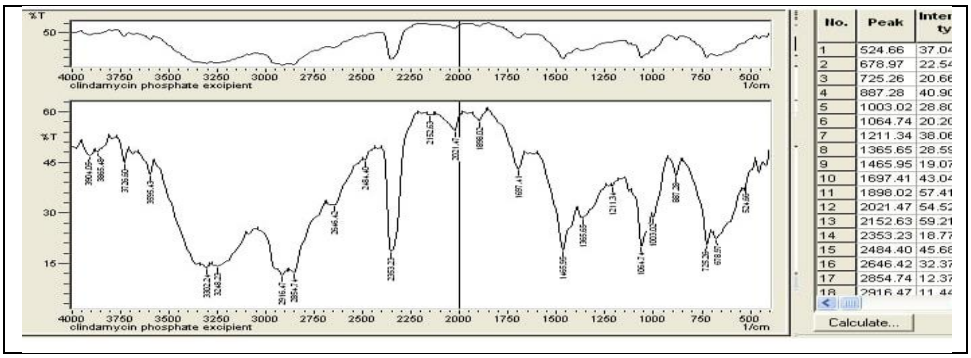


Fig no 3 FTIR of Drug +Excipients

A.Formulation of Clindamycin Phosphate Nanoparticles:

CLP loaded nanoparticles are prepared by the ion tropic gelation nano precipitation method by using the sodium alginate and guar gum natural polymer.clp loaded nanoparticles prepared by varying the concentration of sodium alginate and guar gum .The optimized ratio for the CLP loaded nanoparticles of sodium alginate and guar gum was(1:2).

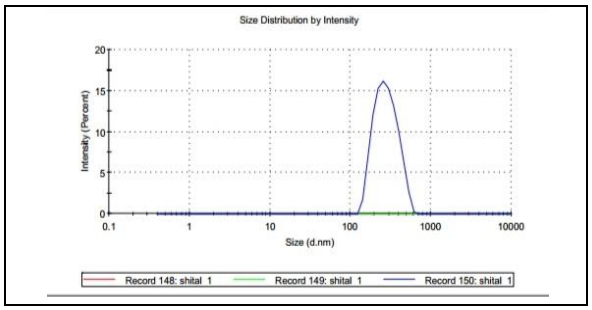
B.Evaluation ofClindamycin Phosphate Nanoparticles:

a) Particle size ,zeta potential and poly-dispersity index[11,12]:

Sr no	Batch no	Zeta potential(Mv)	Particle size(nm)
1	A1	-22.32	217.8
2	A2	-16.88	186.2
3	A3	-14.56	265.2
4	**A4**	**-30. 43**	**242.7**
5	A5	-26.29	297.6
6	A6	-17.91	280.3
7	A7	-15.73	258.7
8	A8	-11.98	303.5
9	A9	-17.57	178.6

Table No: 1 Particle size and zeta potential of CLP loladed nanoparticles .

The ratio of guar gum and sodium alginate in the range of (1:2) shows good particles size



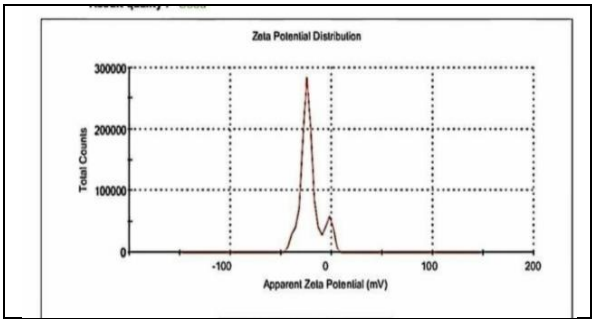


Fig No 4 and Fig No 5 particle size of CLP loaded nanoparticles.(A4)

b) Differential scanning colorimetry (DSC):

The DSC thermo gram for clindamycin phosphate with sodium alginate and guar gum polymer showed an endothermic peak at 209°C.

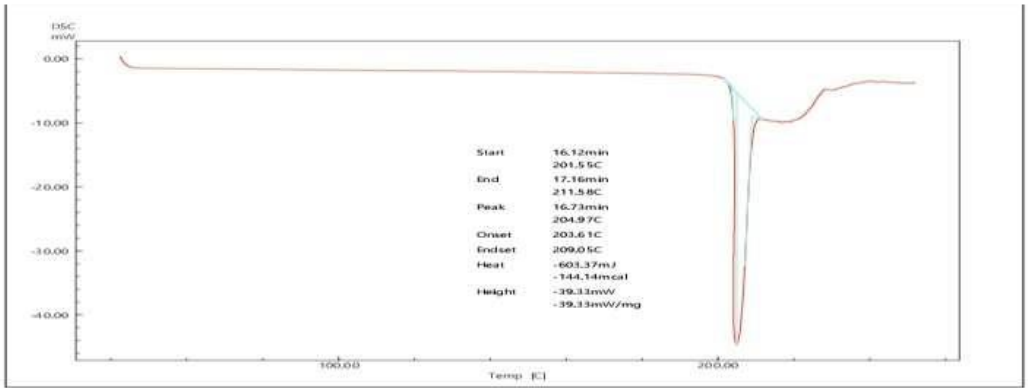


Fig.No 6: .DSC graph of optimized nanoparticle batch. (A4)

c. Scanning electron microscopy (SEM): The SEM image of CLP loaded nanoparticles revealed the cube and somewhat irregular shape and the drug being entrapped in the polymeric material.

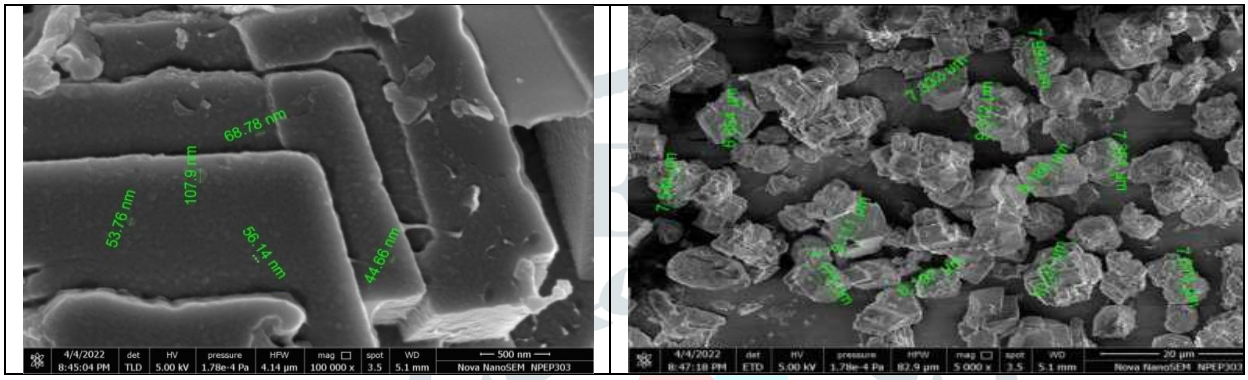


Fig No 7 AND Fig No 8 SEM image of nanoparticles (A4)

d) **Drug Entrapment Efficiency (%):**

Sr no	Batch no	% entrapment efficiency
1	A1	64.06
2	A2	62.0
3	A3	62.45
4	A4	78.23
5	A5	63.25
6	A6	61.23
7	A7	72.23
8	A8	63.01
9	A9	59.09

Table No 2 Entrapment efficiency of CLP loaded nanoparticles loaded formulation

C.Formulation of clindamycin phosphate nanoparticles loaded foam formulation:

Foam is prepared by whipping method

D.Evaluation of clindamycin phosphate nanoparticles loaded foam formulation:

1.Physical evaluation:

The all five batches are evaluated for the physical evaluation .the colour of all five batch was observed white and pH ranges from the 6.1-6.4 .the viscosity of all five batches ranges from the 3535-3640cp.

2. Foam liquid drainage[15,16]–The separation of the liquid due to liquid drainage was observed after 15 min.

3.Foam stability: A cylinder method is used to for following . The higher the FE the more formable is the formulation. The lower the FLS and the higher the FVS the more stable is the produced foam.

So No	FE%	FLS%	FVS	FG fraction (ml)
F1	-26.66	26.66	73.33	-8ml
F2	-13.33	13.33	86.66	-4ml
F3	-6.6	6.6	93.3	-2ml
F4	-15.00	15.00	85	-4.5ml
F5	-16.66	16.66	83.3	-8ml

Table No: 3 stability of foam formulation (f3)

4.*In-Vitro*Diffusion study:

Clindamycin phosphate nanoparticles release studies were carried out during 12 hours as the release of clindamycin phosphate nanoparticles may, therefore, be enhanced through addition of this excipient. Phosphate buffer pH 4.5 containing 25% methanol (v/v) was used as acceptor medium. Clindamycin phosphate nanoparticles foam formulation was released faster from the developed formulation.

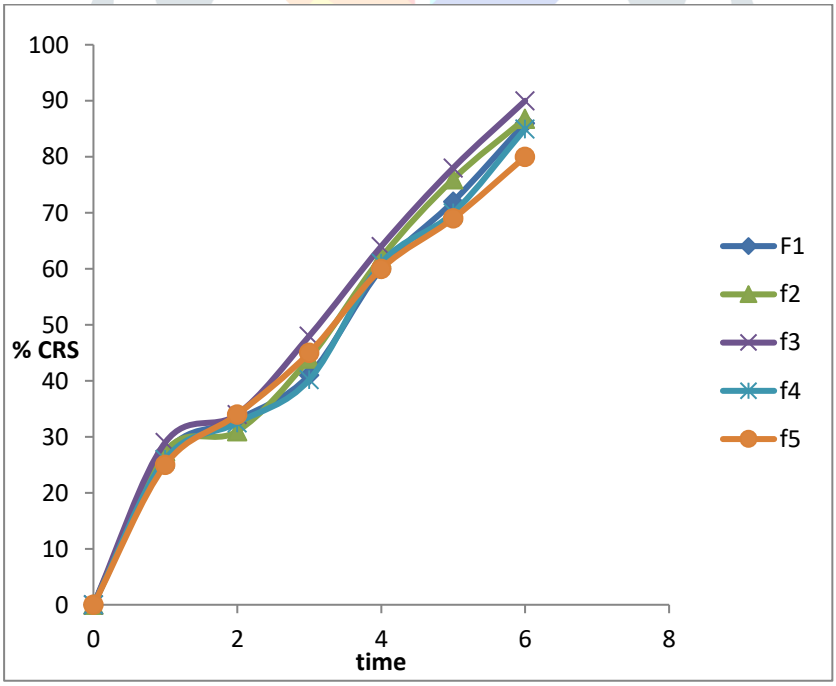


Fig 11. Graph of drug released pattern of (f3)Batch

5.Antibacterial efficacy (in vitro) of selected foam against the *propionibacterium acne*[17].

a).Minimum inhibitory concentration of clindamycin phosphate against the *propionibacterium acne* MTCC No.1951

The *propionibacterium acne* is the prominent species causing acne vulgaris infection of skin .selected formulation was demonstrated good antibacterial efficacy.MIC of clindamycin phosphate 0.2µ/ml concentration.

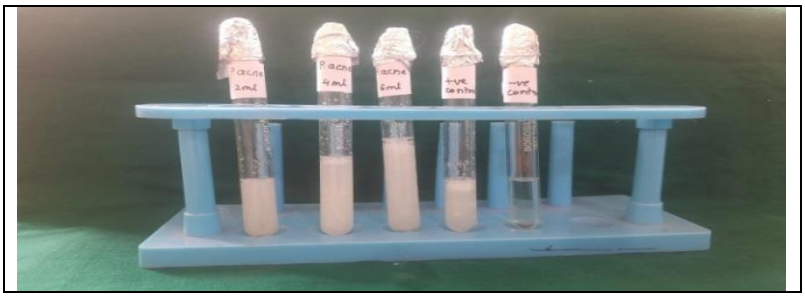


Fig.No.:8 MIC OF CLP against *propionibacterium acne(*f3*)*

b)Zone of inhibition of clindamycin phosphate-

The *propionibacterium acne* is the prominent species causing acne vulgaris infection of skin .Selected formulation was demonstrated good antibacterial efficacy.

Sr.no	Formulation code	Diameter of zone of inhibition(MM)
1.	CLP loaded nanoparticles foam formulation	**75 mm**

Table No.: 5 zone of inhibition of CLP loaded foam formulation (F3)

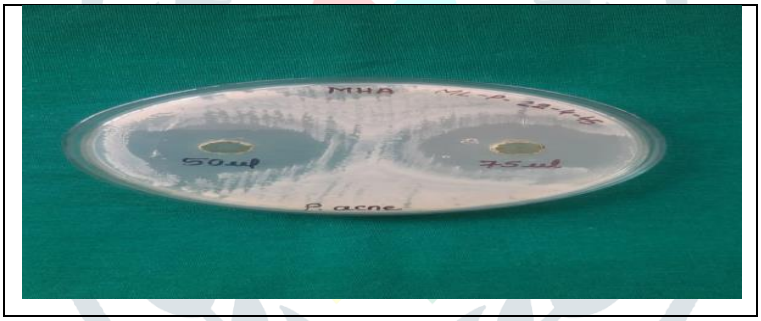


Fig no 9 Zone of CLP against the *propionibacterium acne*(F3)

Fig No.: 10 Zone of CLP against the *propionibacterium acne*(F3)

6. Dermal skin irritation potential (*in-vitro*)[18,19]:

Sr No	Type of sample	Observation	Irritancy score
1	Standard control	No detection of hemorrhage of CAM membrane	0
2	Test formulation	No detection of hemorrhage of CAM membrane	0
3	Positive control	Hemorrhage and dis-coloration CAM membrane	3

Table no 6 skin irritation test (HET-CAM)test. (F3)

Fig no 11 skin irritation test (HET-CAM)test(F3)

Conclusion:

This CLP loaded nanoparticles prepared by ionic gelation nano-precipitation method by using biodegradable polymer such as guar gum and sodium alginate. The guar gum and sodium alginate in the ratio of (1:2) shows the good particle size as the we increase the concentration of guar gum size of nanoparticles increases. The DSC study of polymer with drug shows the endothermic peak at 95.866°C and the SEM study of batch shows the cubic shape nanoparticle and somewhat irregular shape and the drug being entrapped in the polymeric material. The entrapment efficiency of A4 batch is 75% greater than other all batches ,on the basis of particle size ,zeta potential A4 batch shows the greater entrapment efficiency good in particle size and zeta potential. The cetyl alcohol and stearyl alcohol ration up to (60-40) or (80-20) help to maintain the foam characteristics ,make the foam quick breaking as well temperature sensitive low viscosity (3260-10000cps), formulation did not indicate any gross change in any tested parameter for 30 days except liquefaction. The experimental foam formulation containing clindamycin phosphate nanoparticles possessed superior antibacterial activity. The HET-CAM test established non-irritancy of CLP nanoparticles loaded foam formulation .The other advantage of CLP nanoparticle loaded foam formulation is that is dispensed in propellant free pump dispenser ,so itenvironmental safe,reduced chances of contamination, ease of application.

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