



“Fluconazole Mouth Paint for Oral Thrush”

Dr Vijay Hiremath¹. Dr. Purushotham Rao K². Dr Girish Katti³, Dr Rajasree J
Ingin.⁴, Dr Ajay kartik.K⁵, Dr Srikar MAV⁶.,

¹Department of Chemistry., PDA Eng College, Gulbarga.KS,

²CESs college of Pharmacy, Bidar.,KS

³Navodaya Dental College And Hospital.,Raichur.,KS

⁴Gulbarga Institute Of Medical Sciences.,kalaburagi.,KS

⁵Susheela Dental Care, Madhuranagar ,Hyderabad.TS.

⁶Sri dental., Vanasthlipuram., Hyderabad.,TS

ABSTRACT:

Oral mucoadhesive mouth paint preparation was designed and prepared for the treatment of oral candidiasis, where prolonged drug release at the infected area is essential. Fluconazole, a recent triazole derivative having antifungal activity is chosen as the desired drug in this study to formulate mucoadhesive mouth paint. Oral candidiasis is a common infection in debilitated patients, AIDS patients and in persons who administer immunosuppressive drugs. Mouth paints containing 1% fluconazole with hydrophilic polymer NaCMC was prepared and compared with mouth paint prepared without the addition of hydrophilic polymer. The prepared mouth paint formulation was subjected to various evaluation parameters like pH determination, drug content, rheological behaviour, mucoadhesive studies, spreadability and IR spectral analysis. In vitro drug release studies were carried out at salivary pH 6.4 using cellophane membrane as barrier. Stability studies were carried out at different temperature conditions like ambient temp (R. T.), $8 \pm 1^\circ\text{C}$, $45 \pm 2^\circ\text{C}$ at $75\% \pm 5\%$ R. H. (accelerated temperature) 3 months and analyzed at different time intervals for drug content, physical appearance, pH, mucoadhesive strength and spreadability and the prepared formulation was found to be stable. Antimicrobial studies were carried out to ascertain the antifungal activity of prepared mucoadhesive formulation against the pure drug. The test organism *Candida albicans* was a clinical isolate obtained from a diseased patient suffering from oral Candidiasis. In vitro antifungal activity was evaluated using standard Agar cup-plate method by zone inhibition studies. Formulations, containing NaCMC showed good zone inhibition. In vivo oral mucosal skin irritancy tests were carried out using mucoadhesive formulation on lab experimental animals (Rabbits and Guinea-pigs) and on healthy human volunteers. No edema, erythema, inflammation or redness in the mucosal cavity of both animals and human volunteers were observed.

Keywords: Fluconazole, NaCMC, Rheology, Mouth paint.

I. INTRODUCTION

Fungal infections are common in human beings, which are either topical or severe systemic infection. Invasive fungal infections are being identified with an ever-increasing frequency in premature Infants immunocompromised hosts, patients receiving immuno suppressive agents and in those with AIDs. The prevention and treatment of invasive fungal infections have been improved over the last two decades by introduction of new anti-fungal agents such as fluconazole. It is found to be effective in several systemic and topical fungal infections, especially in candidiasis, associated with immuno suppression as in AIDs or cancer therapy.¹ Topical antifungals are usually the drug of choice for localized candidiasis. Traditional methods of drug delivery include mouth washes, mouth paints, gels etc. They suffer from a few practical drawbacks such as they all have relatively short residence times and therefore fail to maintain therapeutic concentration for long enough to affect the microbial population². It is a known fact that for any antimicrobial agent to be effective, maintenance of minimum effective concentration is crucial. If this is not taken care, then along with lack of activity one may face the problem of development of resistance by the organism towards the therapeutic entity. Thus, attempts are being made to design such dosage forms for oral administration which can overcome these above - mentioned drawbacks. **Park and Park**, defined mucoadhesives as a substance that is capable of interacting with biological material and being retained on them or holding them together for extended periods of time. The idea of mucoadhesives was derived from the need to localize drugs at a certain site in the body. Often, the extent of drug absorption is limited by the residence time of the drug at absorption site.³ The objective of the present work was to prolong residence time and increase patient compliance by preparing mucoadhesive mouth paint of Fluconazole, a recent triazole antifungal agent which is less lipophilic and more hydrophilic when compared to other azole antifungal agents. Hydrophilic polymer NaCMC is used as mucoadhesive agent to prolong retention time in oral cavity.

II. MATERIALS AND METHODS

Materials: Drug Fluconazole was gift sample from Glenmark Pharmaceuticals Ltd., Nashik. NaCMC procured from S.D. Fine Chemicals, Ltd., Mumbai cellophane membrane obtained from local market. All other chemicals used were of analytical grade.

Plan of Experimental Work:

Two mouth paint formulations of fluconazole were prepared, containing with and without mucoadhesive polymer sodium carboxyl methyl cellulose following conventional method of mixing the ingredients (Table-1).

Preparation of Mucoadhesive Mouth Paint:

- NaCMC, Sodium citrate and purified water mixed thoroughly and hydrated (for 24 hours).
- Fluconazole was dissolved in 10ml. of ethanol and added to above hydrated base.
- Then add glycerol to the above mixture on stirring to get a homogenous dispersion of drug. Another formulation was prepared without the addition of NaCMC a mucoadhesive polymer.

Characterization of prepared mouth paints:

Drug Content Evaluation:

Drug content was determined by dissolving 2.5 gms. of mouth paint in methanol. After suitable dilution absorbance was recorded by using UV-Spectrophotometer at λ_{max} 261nm. (Table-2)

Determination of pH:⁴

2.5 gm of prepared mouth paint was accurately weighed & dispersed in 25.0ml of purified water (diluted to 10 times), The pH of the dispersion was measured using Digital pH meter.

Spreadability:

For the determination of spreadability, 3 gm. of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1000 gm. weight for 5 mins. Weight (50gm) was added to the pan. The time required to displace upper plate over the lower plate for a distance of 10 cms is noted (Table-2)

Rheological Studies:^{5,6,7}

The viscosity of various formulated Fluconazole Mouth paints were measured by Brook field Viscometer (LV DV-III ultra programmable Rheometer) using spindle CP-52 at varying speed & shear rates from 10, 15, 20, 25 & 30 rpm between 20-60 Sec⁻¹ at room temperature to examine the hysteresis of the rheogram. (Table-3)

Mucoadhesive Strength determination^{8,9}

A sample of 50gms of mouth paint was placed in a 100ml- graduated cylinder for measuring mucoadhesive strength was allowed to penetrate in the sample. The time (sec) the apparatus took to sink 5cms down through the sample. (Table-2)

In vitro drug diffusion studies¹⁰

The permeation apparatus designed as described by Chowdary et. al., & Fites et. al. was employed to study the release of fluconazole from the formulation. Phosphate buffer saline of pH 6.4 using prehydrated Cellophane membrane as barrier. 1 gm. (10mg drug) of prepared mouth paint was taken in donar cell. Samples from receptor compartment (5ml) withdrawn at the interval of 15 min over a period of 180min & assayed for fluconazole at λ_{max} 261nm. The volume with drawn at each time (5ml) was replaced with drug free receptor fluid (PBS of pH 6.4). (Table-4 & Figure-1)

Infrared spectral analysis¹¹

The studies were carried out using IR method with the help of Perkin-Elmer model 983 spectrometer to determine drug excipient interaction. (Figure-2)

Anti-microbial studies^{12,13,14,15}

The prepared mouth paints were evaluated for in vitro antifungal activity using standard Agar cup-plate method. The test organism *Candida albicans* was a clinical isolate obtain from a diseased patient suffering from oral candidiasis from our M.R. Medical College & General Hospital. Gulbarga under the guidance of department staff. The microorganism was collected by sweeping cotton-swab on the tongue of patient and stored this swab in peptone water. Nutrient Agar medium was used for the culture and maintenance of isolated microorganism.

A layer of peptone agar (2ml) seeded with test microorganism was allowed to solidify in Petri dishes by incubating at 37°C for 24hrs. Cups (bore) were made on the solidified agar layer with the help of a sterile cork borer of 5mm diameter. Then approximately 2gm of mucoadhesive mouth paint formulations with drug and pure drug were then poured into the cups and incubated at 37°C for 24-36hrs to observe the extent of zone inhibitions formed in different Petri plates. (Table-5 & Figure-3&4)

Stability studies

The prepared mucoadhesive mouth paint formulations were stored at different temperature condition like ambient temperature, 8±1°C (refrigerator temperature), 45 ± 2°C at 75% ± 5% R.H (condition of accelerated stability testing) for a span of three months & analyzed for drug content, physical appearance, pH spreadability and mucoadhesive strength.

In Vivo studies for oral mucosal skin irritation studies:

The experimental work plan for oral mucosal skin irritation studies was undertaken in:- Laboratory experimental Animals^{16,17}, and Healthy Human Volunteers.

One gram of the prepared 1% Fluconazole mucoadhesive mouth paint was applied to animals oral cavity to test oral skin irritancy effect, in 6 Rabbits and 6 Guinea-pigs. The rabbits were weighing 2.0 to 2.5 kgs and Guinea-pigs 500 to 800 gms. 3 times in 72 hours to each animal and the duration was approximately 24 hours between each application. Irritancy assessment was made by visually examining animals' oral cavity with the focus & magnifying lens to notice any changes in tissues after each application. Then comparison of the photographs of control as against the photographs of oral cavity which have undergone application of mouth paint. Control photographs are the one taken prior to the first application i.e, at zero hr. of the 72 hrs. study time. The oral mucosal skin irritancy study on animals was thus evaluated for any changes like sensitization, oral edema, erythema, redness, inflammation or acute ulcers. (Figure-5 & 6)

Healthy Human Volunteers Studies:

The oral mucosal skin irritation test was performed on six healthy male volunteers by applying 1gm mouth paint formulation (10mg drug). The volunteers were of age group between 23-28 years and weighing 55 to 65 Kgs. All the volunteers were regular in their oral hygiene regime and usually brushed and gargled twice a day. The legal ethical committee approved the study and each subject gave written informed consent before indulging in the study. The volunteers were fasted without food and water for atleast 3hrs before each application. The volunteers abstained from taking any medicines/ alcoholic drink / chewing tobacco for over 30hrs at the start of test and during the entire 72hrs. of study. During other times the regular food and water was served / allowed. The prepared mucoadhesive mouth paint formulation of 1gm for each application (10mg drug) was applied with the aid of brush on the Dorsal part of tongue, hard and soft palate and buccal region of the mouth of volunteers for a period of 72hrs (3 days) study with a duration of approximately 24hrs per application. Volunteers were parted into 3 groups involving 2 volunteers in each group. The formulation was applied on each group of two volunteers for the study. The preparations were allowed to retain for overnight restricting the volunteers from taking any liquid / solid intake.

Oral Mucosal Irritancy Assessment:

It was performed primarily by examining each volunteer oral cavity barely with naked eyes using focus and magnifying lens to notice any changes in tissues after application of formulations. Then photographic imaging of oral cavity of human volunteers was taken out after subsequent application for 72hrs i.e., at completion of study period and these images were compared to determine the difference with the images taken at zerohr. of study i.e., prior to first application of formulation. Moreover, mucosa irritation was evaluated by questioning the human volunteers at regular interval of time about the feeling of irritancy, which appears to be highly subjective for the study. Finally, the oral mucosal skin irritancy was evaluated for any changes like oral erythema, inflammation, redness, haemorrhagic lesions or acute painful ulcers (canker sores).

III. RESULTS AND DISCUSSION:

In the present piece of investigation, the mucoadhesive mouth paint preparations of Fluconazole can be designed using hydrophilic polymer like NaCMC for the treatment of Oral thrush. During our physico-chemical evaluation studies the formulation was found to have good spreadability and mucoadhesive strength. The drug content for MMP was 100.30%. In our present investigation of in vitro drug release studies, the mucoadhesive mouth paint showed optimum release of 75.77% in 3 hrs. as against conventional mouth paint formulation showed 84.14% in 75 minutes. The rheological behaviour of both mucoadhesive mouth paint and conventional mouth paint were studied. Mucoadhesive mouth paint data shown shear thinning (pseudoplastic) behaviour, where there is decrease in viscosity by increasing shear rate. This shear thinning behaviour is a desirable property for topical preparations, as they should be thin during application and thick otherwise. The formulation mucoadhesive mouth paint showed good mucoadhesive strength (92 secs) when compared to conventional mouth paint formulation (11 secs), which measure the viscosity at physiological temperature.

During microbiological investigation against the causative organism collected from the patient of Oral thrush, the hydrophilic polymer containing NACMC, formulation showed good zone inhibition when compared with pure drug. IR studies revealed that there is no drug excipients interaction. The undisturbed peaks of pure drug at 1646.11 cm^{-1} and 1417 cm^{-1} is due to C=C and C=N stretching vibrations and at 1232 cm^{-1} aromatic C-F stretching vibration confirm the undisturbed drug in formulation. In our present investigation of stability studies, the prepared formulation did not segregate, ferment or physically deteriorated during storage & use at different temperature conditions for a period of 3 months. The formulation did not undergo phase separation or gassing fermentation or otherwise deterioration aesthetically. In vivo studies were carried out to study the oral mucosal skin irritation on both laboratory experimental animals (Rabbits & Guinea-Pigs) in our animal house and on Healthy Human volunteers with the help of staff ENT Dept. of our medical college. The studies revealed that the usage of 1% Fluconazole mucoadhesive mouth paint formulation did not produce oral mucosal skin irritancy. On observation no edema, erythema, inflammation or redness seen in the mucosal cavity of both animals & Human volunteers indicating formulations high compliance with oral mucosal surface, thereby passing the test of compatibility studies. The present study revealed that the prepared Fluconazole mucoadhesive mouth paint formulation with more retentive time in oral cavity will be useful than conventional mouth paints, which have short retentive time.

IV. CONCLUSION:

The results of present study on mucoadhesive mouth paint designed for the treatment of Oral thrush will be useful for drug industry to formulate localized drug delivery to benefit the patients suffering from Oral thrush of all ages and sex.

ACKNOWLEDGEMENTS:

The authors are thankful to Principal, HKE's College of Pharmacy, ethical committee of M.R. Medical College, Gulbarga for in-vivo studies in animals and Dr.Mallikarjun Reddy, Professor and H.O.D., Department of ENT, M.R. Medical College and General Hospital, Gulbarga for his guidance and Help in Oral mucosal skin irritation studies and for providing candida albicans microorganism from Oral thrush patients.

BIBLIOGRAPHY:

- [1] Devi. V.K. et. al. "Orodispersible Fluconazole Tablets-Preparation and Evaluation". Indian Drugs. Vol. 23. No. 7. July 2006; p.548-52.
- [2] Vyas S.P. and Khar Roop K., Controlled drug delivery- concepts and advances. Vallabh Prakashan, Delhi, 1st edition. 2002; p.292-97.
- [3] Jain N.K. Controlled and Novel Drug Delivery. Chapter 16- Mucoadhesive Drug Delivery. Khar Roop K., Ahuja Alka and Ali Javed. CBS publishers and distributors, New Delhi. 2005; p.353-76.
- [4] Jelvehgari Mitra et. al. "Mucoadhesive and Drug Release properties of Benzocaine Gel". Iranian Journal of Pharmaceutical science. Autumn 2006: 2 (4): p.185-194.
- [5] Lieberman A. Herbert et. al. "Pharmaceutical Dosage Forms" Chapter-9 Rheology of Dispersed systems. Deem Donald E. Marcel Dekker, Inc. U.S.A., Vol 1, 1998; p.367-422.
- [6] Rawlins E.A. "Bentley's Text book of Pharmaceutics." Chapter 9-Rheology. Bailliere Tindall, London. 8th edition 1995; p.123-39.
- [7] Alfred Martin. "Physical Pharmacy"-Rheology. 4th edition B.I. Waverly publications, New Delhi. 1997; p.453-73.
- [8] Yong Chul Soon et. al. "Effect of sodium chloride on the Gelation temperature, gel strength and bioadhesive force of Poloxamr gels containing diclofenac sodium". Int. J. Pharm. 1998; 165; p.33.
- [9] Gonjari I.D. and Kasture P.V. "Temperature Induced in situ mucoadhesive gel of Tramadol hydrochloride for Nasal Drug Delivery." Journal of Pharmaceutical Research. Vol. 6, No.2, April 2007; p.89-93.
- [10] Chowdary K.P.R. and Kumar P.A. "Formulation and evaluation of Topical Drug Delivery systems of Ciprofloxacin." Indian J. Pharm. Sci., 1996, Vol 58, No.2; p.47-50.

- [11] Dash Alekha K. and Elmquist William F. "Fluconazole" Analytical profiles of Drug substance and Excipients." Academic press, Inc. London. Published by Elsevier. Edited by- Brittain Herry G. Vol 27; 2008; p.92.
- [12] Sahoo S. et. al. "Antifungal activity of *Hybanthus Enneaspermus* against selected Human Pathogenic fungi". Indian Drugs. Vol. 44, No.5, May 2007; p.352-56.
- [13] Madhusudhan B. et. al. "Development and evaluation of Antifungal activity of o/w type creams containing solid dispersion of Clotrimazole" Indian J. of Pharm. Sci., Nov.-Dec.1999, Vol 61; p.346-349.
- [14] Mehra R. Gilhotra and Mishra Dina N., "Preparation, characterization and In vitro Dissolution studies of surface cross-linked ocular Films of Gatifloxacin." Ethiop Pharm. Journal. Vol-28, 2008; p.1-8.
- [15] Ganguli Subrana et. al. "Study of antifungal activity of some 3-(arylideneamino)-2-phenyl quina zoline-4(3h)-ones". Adv. Pharmacol. Toxicol. Vol.9 (3) 2008, p. 59 - 61.
- [16] Jain N.K. "Controlled and Novel Drug Delivery." Chapter 3-Oral Transmucosal Drug delivery, Devarajan P.V. and Adani M.H., CBS publishers and Distributors, New Delhi. 1st edition 1997; p.59-60.
- [17] Jain N.K. "Controlled and Novel Drug Delivery". Chapter-5 Transdermal Drug Delivery. Mishra A.N. CBS Publishers and Distributors, New Delhi. 1st edition, 1997; p.125Ap.

Table-1: Formula used to prepare mouth paints

| Sl. No. | Ingredients | CMP* | MMP* |
|---------|----------------|---------|---------|
| 1. | Fluconazole | 1.0 gm. | 1.0 gm. |
| 2. | Sodium citrate | 1.0 gm. | 1.0 gm. |
| 3. | NaCMC | - | 1.0 gm. |
| 4. | Glycerol | 60 ml. | 60 ml. |
| 5. | Alcohol | 10 ml. | 10 ml. |
| 6. | Water upto | 100 ml. | 100 ml. |

* CMP - Conventional mouth paint.

* MMP - Mucoadhesive mouth paint.

Table-2: Physico-chemical parameters of prepared mouth paints

| Sl. No. | Parameters | CMP | MMP |
|---------|-----------------------|-------------|------------|
| | Drug Content | 100.60% | 100.30% |
| | pH | 6.90±0.043 | 7.29±0.007 |
| | Spreadability | 10.14±0.034 | 8.11±0.460 |
| | Mucoadhesive strength | 11.00±0.480 | 92.0±0.250 |

* Each reading is a mean of three replicates.

* Each sample of 1 gm. Paint contains 10mg. of drug.

Table-3: Viscosity data of prepared Conventional Mouth Paint and Mucoadhesive Mouth Paint formulations

| CMP | | | | | MMP | | | | |
|-----------------|-------------|---------------------------------|------------|-----------------------|-----------------|-------------|---------------------------------|------------|-----------------------|
| Viscosity (CPS) | Speed (rpm) | Shear Rate (Sec ⁻¹) | Temp. (°C) | Time Intervals (Sec.) | Viscosity (CPS) | Speed (rpm) | Shear Rate (Sec ⁻¹) | Temp. (°C) | Time Intervals (Sec.) |
| 721.40 | 10 | 20 | 28.60 | 30 | 1251.10 | 10 | 20 | 28.8 | 30 |
| 633.50 | 15 | 30 | 28.70 | 30 | 753.40 | 15 | 30 | 28.8 | 30 |
| 470.50 | 20 | 40 | 28.60 | 30 | 586.30 | 20 | 40 | 28.8 | 30 |
| 357.80 | 25 | 50 | 28.60 | 30 | 354.90 | 25 | 50 | 29.0 | 30 |
| 304.30 | 30 | 60 | 28.70 | 30 | 251.40 | 30 | 60 | 29.0 | 30 |

Table-4: In vitro drug release of prepared Conventional Mouth Paint and Mucoadhesive Mouth Paint formulations

| Sl. No. | CMP | | | MMP | | |
|---------|------------|----------------------------|-----------------------------|------------|----------------------------|-----------------------------|
| | Time (min) | % Cumulative drug released | % Cumulative drug remaining | Time (min) | % Cumulative drug released | % Cumulative drug remaining |
| 1. | 0 | 0.0 | 0.0 | 0 | 0.0 | 0.0 |
| 2. | 15 | 29.12 | 70.88 | 15 | 16.3 | 83.7 |
| 3. | 30 | 43.47 | 56.53 | 30 | 23.91 | 76.09 |
| 4. | 45 | 62.83 | 37.17 | 45 | 30.55 | 69.45 |
| 5. | 60 | 73.39 | 26.61 | 60 | 37.57 | 62.43 |
| 6. | 75 | 84.14 | 15.86 | 75 | 43.20 | 56.8 |
| 7. | | - | - | 90 | 49.47 | 50.53 |
| 8. | | - | - | 105 | 55.57 | 44.43 |
| 9. | | - | - | 120 | 60.96 | 39.04 |
| 10. | | - | - | 135 | 66.01 | 33.99 |
| 11. | | - | - | 150 | 69.65 | 30.35 |
| 12. | | - | - | 165 | 72.72 | 27.28 |
| 13. | | - | - | 180 | 75.77 | 24.23 |

Table-5: Antimicrobial Studies Data

| Formulation Code | Zone inhibition (mm) after 36 hrs. | | | |
|------------------|------------------------------------|--------|--------|------------|
| | Zone-1 | Zone-2 | Zone-3 | Mean |
| MMP | 22.0 | 23.0 | 25.0 | 23.33±1.52 |
| Pure Drug | 25.0 | 26.0 | 26.0 | 25.6±0.577 |

* Each reading is a mean of 3 replicates

* All above formulation contain 1% fluconazole.

Fig-1: In vitro drug release of prepared Conventional Mouth Paint and Mucoadhesive Mouth Paint formulations

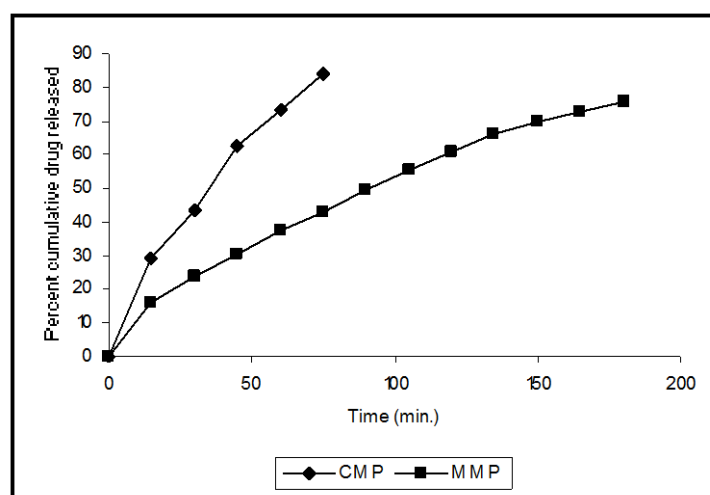


FIG-2

Comparative Study of I.R. Spectra of Pure Drug (Fluconazole) with MMP formulation containing fluconazole with other excipients

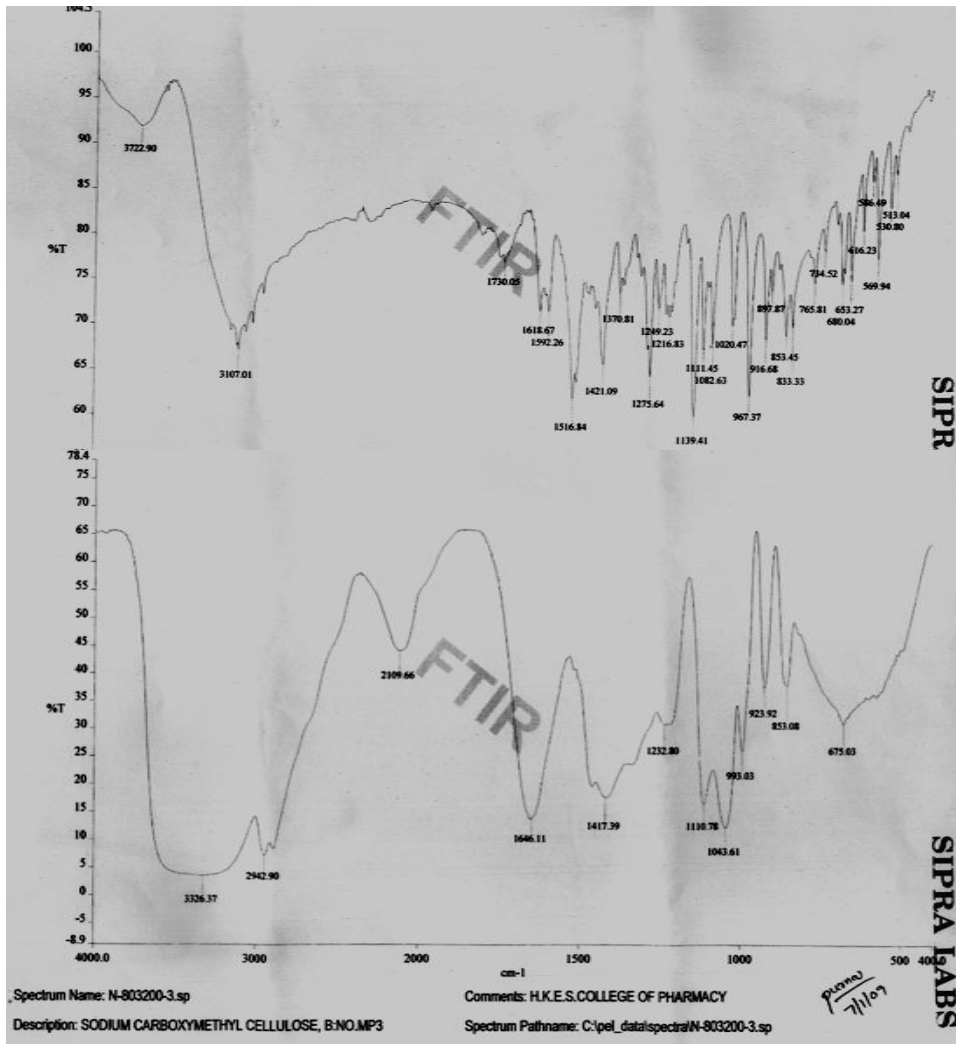


FIG-3: PHOTOGRAPHS OF ANTI-MICROBIAL STUDIES SHOWING THE COMPARATIVE ZONE INHIBITION OF PURE DRUG AS AGAINST DRUG IN FORMULATIONS



Pure Drug



Formulation MMP

FIG-4: HISTOGRAM SHOWING COMPARATIVE ZONE INHIBITIONS OF PURE DRUG AND DRUG IN MMP FORMULATION

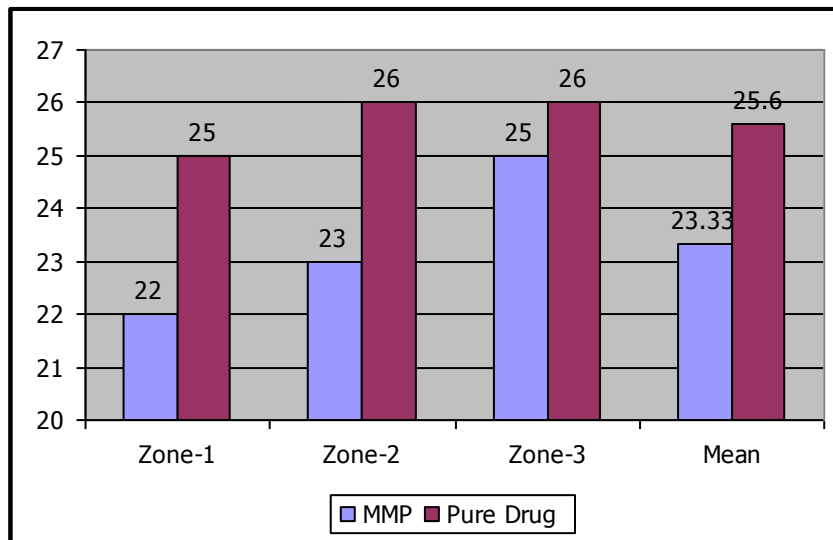


FIG-5: SKIN IRRITATION TEST OF ORAL MUSCOSA IN RABBITS



Before Application



After 72 hrs. of Application

FIG-6: SKIN IRRITATION TEST OF ORAL MUSCOSA IN GUINEA PIGS



Before Application



After 72 hrs. of Application

FIG-7: SKIN IRRITATION TEST OF ORAL MUCOSA IN HUMAN VOLUNTEERS



Before Application



After 72 hrs. of Application