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Formulation and Evaluation of Ivermectin Gel For Treatment of Pediculosis Capitis

Suchita Dhamane[1]*, Reshma Ughade[1], Vaishali Potnis[1], Geet Asnani[1], Aditi Kulkarni[1]
1.JSPM's Jaywantrao Sawant college of Pharmacy and Research, Hadapsar, Pune, 411 028, Maharashtra, India.

ABSTRACT

Infestation of the head louse caused by *Pediculus humanus capitis* is an important public health problem worldwide. FDA has approved Ivermectin as an antilice drug in 2012. Commercially Ivermectin is available in the form of Sklice lotion in US market. Hence, in the present work an attempt was made to formulate hair gel of Ivermectin for better patient compliance and to avoid the side effects related with other formulations. The gels of 0.5% Ivermectin were prepared using different gelling agents such as carbopol 934, hydroxypropyl methylcellulose K4M, hydroxyethyl cellulose 250 HHX. Glycerin and propylene glycol were used as a humectants having plasticizer activity. The formulations were characterized for pH, viscosity, spreadability, extrudability, drug content, *in vitro* drug release, antilice activity, skin irritation study and stability studies. Among the selected formulations, formulation containing 2% HEC 250 HHX and 15% propylene glycol showed better viscosity, spreadability, extrudability and *In vitro* drug release as compared to other formulations. It showed excellent antilice activity and no skin irritation was observed. It was found to be stable at 25°C/60% and 40°C/75% RH over a period of 45 days. These results suggest the feasibility of the topical gel formulation of Ivermectin for the treatment of pediculosis.

Keywords: *Pediculus humanus capitis*, Ivermectin, antilice, Carbopol 934, HPMC K4M, HEC 250 HHX.

*Corresponding Author Email: suchi266@gmail.com
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INTRODUCTION

Head lice are an emerging social problem, not only in economically poor countries but also in practically all other societies[1]. Hundreds of millions of human infestations with head lice are thought to occur annually (Taplin & Meinking, 1987). Numbers have increased worldwide since the mid-1960s and an estimated 40 – 45% people, mainly children, undergo some sort of treatment annually. Every year 6-12 million people in the United States are infested by head lice (Pediculosis). In Australia, head lice infestation is an important public health problem and up to a third of students are infested in some schools (Speare & Buettner, 1999).

Head lice are tiny six-legged insects that cling to the scalp and neck and feed on human blood. They feed by injecting saliva with vasodilatory properties into the scalp to draw blood and can move quite quickly in the hair. The head louse is a grey-white animal about 2-3 mm in length (about the size of a sesame seed). The life span of the female louse is about one month. Head-lice transmission is most commonly via direct head-to-head contact. Indirect transmission occurs by using infested combs, brushes or towels, wearing clothing, such as hats, scarves, coats, sport uniforms, or hair ribbons worn by an infested person. The head lice cause substantial distress due to bite reactions, scalp, excoriation, sleep loss, local erythema, scalp impetigo, papules and cervical and occipital lymphadenopathy, itching, probable secondary infections, pruritis and conjunctivitis are also common manifestations. Although the symptoms are relatively mild, infestation by *P. humanus capitis* has resulted in various social, mental and economic problems[2, 3, 4].

The increasing global prevalence of pediculosis and accompanying increased awareness highlight the need to approach alternatives to head lice control more seriously. Current topical head lice treatments include synthetic pediculocides like organochlorine (DDT and lindane), organophosphates (Malathion), carbamate (carbonyl), pyrethrin, pyrethroid (Permethrin) and avermectin (Ivermectin-originated from streptomyces avermitilis) insecticides. Several of the common antilouse products have lost at least in part their efficacy due to increasing resistance of lice against insecticides such as permethrin. Other compounds, like lindane, were redrawn or banned in market due to high toxicity. Some recently developed products are based on dimethicones or cyclomethicones and turned out to be easily inflammable[2, 3,4].

Gels are defined as “semisolid system in which a liquid phase is constrained within a polymeric matrix in which a high degree of physical and chemical cross-linking introduced”. Hydrogel is a network of polymer chains that are water-insoluble, sometimes found as a colloidal gel in which

water is the dispersion medium. Hydrogels are superabsorbent (they can contain over 99% water) natural or synthetic polymers. Hydrogels have played a key role in drug delivery technology[6].

Ivermectin is the most common avermectin. It is a broad spectrum antiparasitic drug. It is approved by FDA as an antilice agent in Feb 2012. Ivermectin binds selectively and with high affinity to glutamate-gated chloride ion channels in invertebrate muscle and nerve cells of the microfilaria. This binding causes an increase in the permeability of the cell membrane to chloride ions and results in hyperpolarization of the cell, leading to paralysis and death of the parasite[5]. Commercially it is available in the market in form of lotion as a Sklice lotion (0.5 %) for the treatment of pediculosis.

In present work an attempt was made to formulate and evaluate topical antilice hair gel of 0.5% Ivermectin by using different gelling agents such as Carbopol 934, HPMC K4M, HEC 250 HHX. For development of gel other additives such as PEG 400, propylene glycol, glycerin, menthol, triethanolamine and water were used. The objective of the present work formulation was to overcome the problems associated with other formulations and to avoid drying of the gel by the use of humectant, to reduce the treatment time and to enhance the antilice activity.

MATERIALS AND METHOD

Materials:

Ivermectin was procured as gift sample from Nulife Pharmaceuticals, Pune. Carbopol 934 (Emco Industries, Mumbai), HEC 250 HHX (Macleods pharmaceuticals ltd., Mumbai), HPMC K4M (Sahyadri Chemicals, Islampur), PEG 400 (SD Fine chemicals), Propylene glycol (Sahyadri laboratory chemicals, Pune), Glycerin (SD Fine chemicals, Mumbai), Potassium dihydrogen Phosphate (Pure chem. Lab. Pune), Ethanol, Menthol used were of analytical grade.

Characterization of Ivermectin

UV-Visible spectrophotometry

Stock solutions of 10 µg/ml of ivermectin was prepared in ethanol and it was scanned in the range of 400 to 200 nm and respective λ max values were reported. Calibration curve of 2 to 14 µg/ml solution at this λ max value was prepared. Similar procedure was carried for UV spectrum & calibration curve in Phosphate buffer saline (pH 6.8): Methanol (95%) (6:4).

Drug-excipient compatibility study

The physicochemical compatibility between Ivermectin and formulation excipients used in the gel formulations was studied by using Fourier transform infrared spectroscopy. For this, the physical mixtures were prepared (quantity as per formulation required). The physical mixtures and

potassium bromide were mixed in a 1: 3 ratio and kept in sample cell, the cell was then fitted on sample holder and IR spectra were recorded with pure potassium bromide over 4000-400 cm^{-1} after baseline correction was made.

Formulation of anti-lice gel

Individual gelling agents of weighed quantity were soaked overnight in the measured quantity of purified water. Measured quantity of methyl paraben, propyl paraben and weighed quantity of polyethylene glycol were dissolved in about 35 ml of water in beaker. The above mixture was stirred at high speed using mechanical stirrer and added to the beaker containing gel base. Crushed menthol was incorporated slowly in above dispersion after smooth dispersion is obtained. Ivermectin was dissolved in propylene glycol or glycerine and added to the above mixture. Then triethanolamine (gelling agent) was added slowly while stirring till to attain gel structure of acceptable pH. The details are shown in table no. 1

Table 1: Composition of ivermectin gels (% w/w)

Name of additive	Formulation code								
	IGC1	IGC1G2	IGC1P3	IGH2	IGH2G3	IGH2P3	IGN2	IGN2G2	IGN2P3
Ivermectin	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Carbopol 934	0.6	0.6	0.6	_	_	_	_	_	_
HPMC K4M	_	_	_	3.5	3.5	3.5	_	_	_
HEC 250 HHX	_	_	_	_	_	_	2	2	2
PEG 400	10	10	10	10	10	10	10	10	10
Glycerin	_	10	_	_	10	_	_	10	_
Propylene glycol	_	_	15	_	_	15	_	_	15
Menthol	1	1	1	1	1	1	1	1	1
Methyl paraben	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075	0.075
Propyl paraben	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Triethanolamine	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Purified water q.s to 100	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Evaluation of topical Ivermectin gel:

Physical examination:

The prepared gel formulations were examined for their color, odor, homogeneity and presence of air bubbles.

pH:

The pH of gel formulations was determined by using digital pH meter. The pH meter was calibrated before each use with standard pH 4, 7 and 9 buffer solutions. About 1gm of gel was

dissolved in 50 ml of distilled water. The measurement of pH of each formulation was made in triplicate and average values were calculated.

Viscosity determination:

The gel formulations were assessed for viscosity (cps) using a Brookfield viscometer (Brookfield LVDV, spindle no. S 64) at 25°C-28°C. For this approximately 50 gm of sample gel was placed in the 25ml beaker and the spindle was dipped into it. The spindle was rotated in the gel at 10 rpm and the corresponding viscosity (in cps) and torque (in %) values were noted.

Spreadability:

Spreadability is determined by apparatus suggested by Mutimer et al (1956) which is suitably modified in the laboratory and used for the study. It consists of a wooden block, which was provided with a pulley at one end. A ground glass slide was fixed on this block. An excess of gel (about 2 gm) was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide with the same dimensions of fixed ground slide and provided with the hook. A 1 Kg weight 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 the edges. The top plate was then subjected to pull of 80 gms, with the help of string attached to the hook and then the time (in seconds) required by the top slide to cover a distance of 7.5 cm be noted[9]. Spreading ability was calculated by using the formula:

$$S = M. L /T$$

Where, S- Spreading Coefficient, M = weight tied to upper slide

L = length of glass slides T = time taken to separate the slides.

Extrudability (Tube Test):

The method adopted for evaluating gel formulation for extrudability was based upon the quantity in percentage of gel extruded from lacquered aluminum collapsible tube on application of weight in grams required to extrude at least 0.5 cm ribbon of gel in 10 seconds[7].

Drug content:

The quantity of gel equivalent to 10 mg of Ivermectin was dissolved in 100 ml ethanol. From this solution, 1 ml samples were withdrawn and diluted to 10 ml with ethanol. These samples were analyzed spectrophotometrically at a wavelength of 246 nm and concentration of Ivermectin in each sample was estimated from previously prepared standard curve[8].

Drug Content = (Concentration × Dilution Factor × Volume taken) × Conversion Factor

***IN VITRO* DIFFUSION STUDY USING SYNTHETIC MEMBRANE:**

Preparation of the cellophane membrane for the diffusion studies:

The cellophane membrane approximately 25 cm × 2 cm was taken and washed in the running water. It was then soaked in distilled water for 24 hours, before used for diffusion studies to remove glycerin present on it and was mounted on diffusion cell for further studies.

Diffusion study :

Drug release (*in vitro*) from gel was estimated using Franz Diffusion cell through cellophane membrane which was placed between donor and receptor compartments of the diffusion cell. The quantity of gel equivalent to 10 mg of Ivermectin was spread uniformly on the dialysis membrane. Phosphate buffer solution (pH 6.8): methanol (9:1) was filled in the receptor compartment. 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°C. A similar blank set was run simultaneously as a control. Sample (2 ml) was withdrawn at suitable time intervals and replaced with equal amounts of fresh media. Samples were analyzed spectrophotometrically at 247 nm and the cumulative % drug release was calculated. The difference between the readings of drug release and control was used as the actual reading in each case.

Antilice Activity:

Nowadays Filter paper assays in petridishes are common bioassay method to determine the antilice activity, which provides informative and comparable results[14]. Human head lice *Pediculus humanus capitis* were collected by combing the soiled, unwashed and uncombed hairs of 4-5 infected children between the age group of 8 – 12 yrs, with the consent of their guardians, residing at Pune district. Adult lice were placed in small wide mouth plastic containers (50ml polypropylene containers) containing 1.5 cm hair tufts of a same volunteer. The mouth was covered with nylon mesh (1strand/mm) to permit ventilation for lice. The *in-vitro* tests were started within 15 min after collection of lice. After careful selection of lice under a dissecting microscope, a filter paper discs (Whatman No 1; 9-cm diameter) coinciding with internal diameter of petri dish were cut and placed in petridishes. The activity is done in triplicate. At each time 10 lice were released in the petridish of each group and impregnated with the formulations and maintained at conditions for lice of 28±2 °C, 60±20% relative humidity (RH).

The antilice activity was performed in four groups,

Group 1: Purified water

Group 2: Non medicated gel base

Group 3: Ivermectin (0.5%) gel

Group 4: Standard marketed gel

The lice were judged as dead if there were no vital signs such as movements of antennae or minimal leg movements (with or without stimulation by a forceps). Death was defined as lack of movement of limbs and gut, and failure to respond when the legs were stroked with forceps[10, 11, 14, 15]. Data obtained was analyzed by one way ANOVA followed by Tukey Kramer test.

Accelerated stability Studies :

The hair gel formulation IGN2P3 was subjected to stability study as it exhibited good drug release when compared to others and showed excellent antilice activity. The gel formulation IGN2P3 which was filled earlier in collapsible tube was labeled and stored at 25°C/60% relative humidity (RH) and 40°C/75% RH for a period of 45 days. Samples were withdrawn at time intervals of 15 days and evaluated for physical appearance, pH, rheological properties, drug content and drug release.

Skin irritation studies *(in vivo):*

Gels should not produce skin irritation when applied topical drug delivery system. Hence, skin irritation study was performed. To test the irritancy potential of prepared Ivermectin gel, healthy male Wistar rats of weight 150 - 200g were used. Topical dose applications were divided into four groups, Group I = Control, Group II = Gel base treated control, Group III = Negative control, Group IV = Test formulation. Animals were randomly placed in cages upon receipt and then randomized according to the body weights. The animal backs were carefully shaved using sterilized shaving blade to remove the fur. The selected animals were grouped into 4 groups and were marked with identification codes using diluted picric acid solution (1.2%w/v). Circular areas of 2.54 cm (1 inch diameter) were marked on the back of each animal using marker ink one spot on right side and one spot on left side of vertebral column. The spot on right side was abraded using shaving blade and the spot on left side was kept intact. About 1gm of the selected gels was applied on marked spots using absorbent cotton wool. The toxic manifestations if any on the skin were then assessed by observing these skin areas at pre-selected time intervals after treatment e.g. 1 hr, 4 hrs, 12 hrs, 24 hrs, 48 hrs and 72 hrs. The observations were recorded as numerical scores as follows for each animal.

0 = no visible reaction

1 = mild erythema

2 = intense erythema

3 = intense erythema with edema

4 = intense erythema with edema and vesicular erosion

The scores for treatment group and control group animals were then compared[12, 13].

RESULTS AND DISCUSSION

In present work, attempt was made to formulate and evaluate topical gel drug delivery systems for the treatment of Pediculosis. The ultimate aim was to overcome the problems associated with other formulations and to avoid drying of the gel, to reduce the treatment time and to enhance the antilice activity. The UV spectrum (λ_{max}) of Ivermectin in ethanol (99.9%) indicated λ max at 246 nm. The λ_{max} of Ivermectin in phosphate buffer saline (pH 6.8): Methanol (99%) was 247 nm. The standard calibration curve of pure Ivermectin in ethanol and phosphate buffer saline (pH 6.8): Methanol (99%) at 246 nm and 247 nm was found to be linear over the range of 2 -12 µg/ml as given in figure 1 and figure 2 respectively

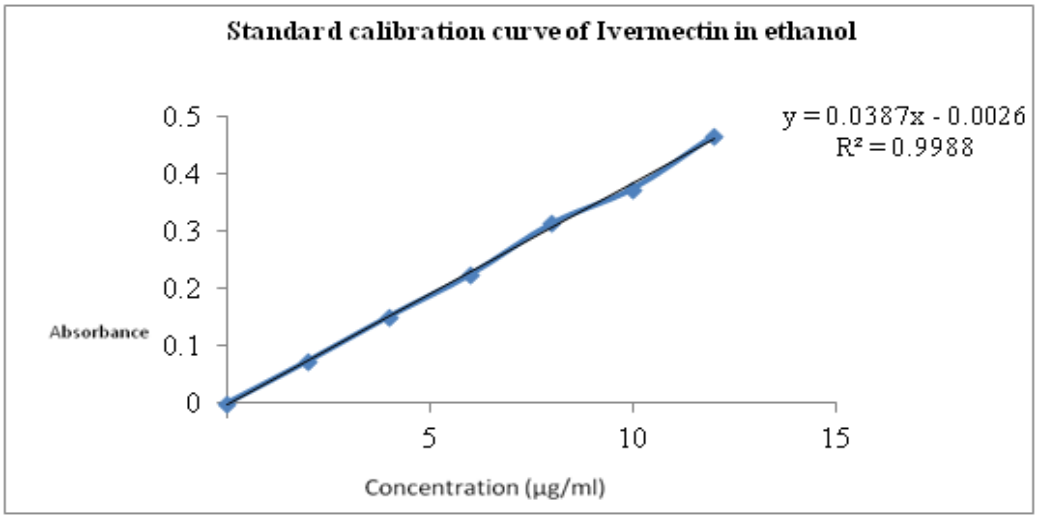

Figure 1: Calibration curve of Ivermectin in ethanol

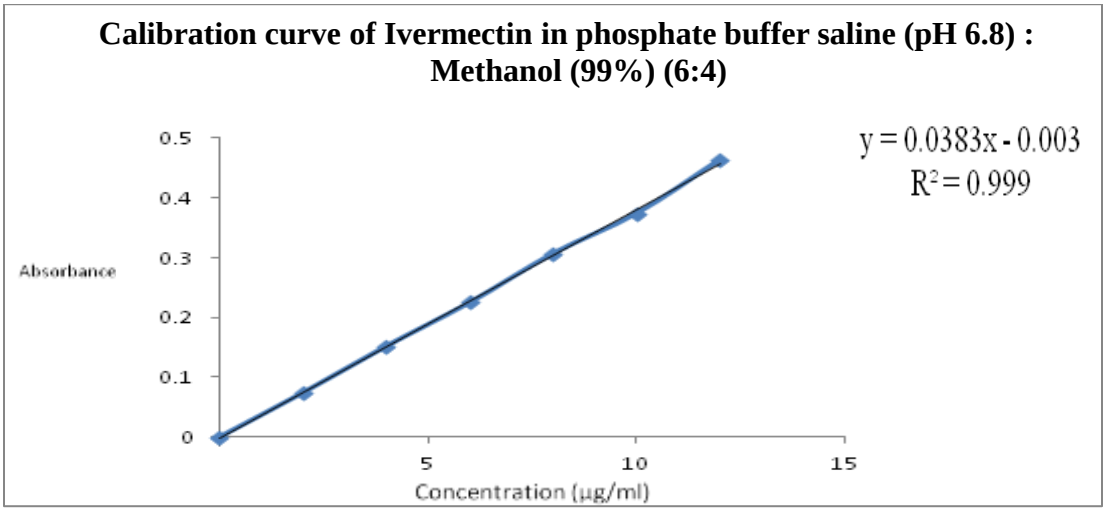

Figure 2: Calibration curve of Ivermectin in phosphate buffer saline (pH 6.8) : Methanol (99%) (6:4)

Drug-Excipient compatibility study

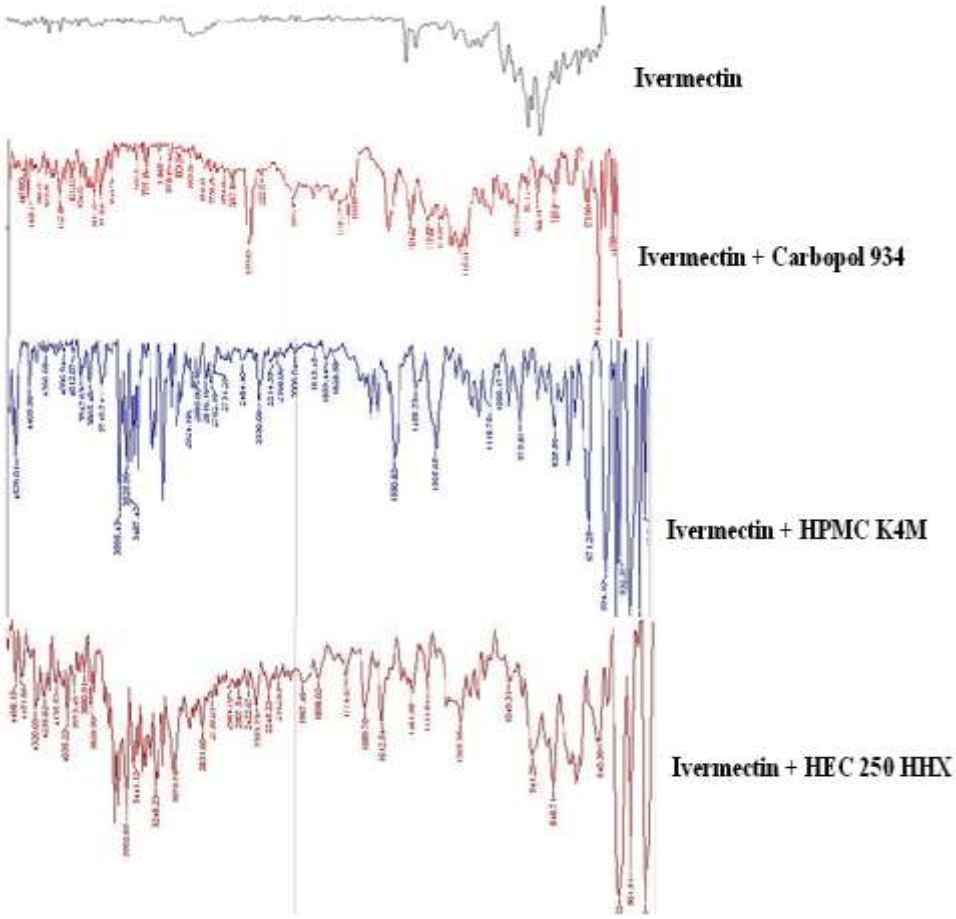

Figure 3 :Drug- Excipient compatibility study

Drug-excipients compatibility was carried out by FT-IR analysis. FT-IR study showed that there was no major change in the position of peak obtained in the ivermectin alone and in a mixture of drug with excipients, which shows that there was no interaction between drug and excipients.

EVALUATION OF IVERMECTIN GEL

Physical examination –

All formulations were found off white, translucent, non gritty except IGC1, free from air bubbles and with suitable homogeneity and consistency.

pH:

All the gel formulations formulated using different gelling agents were in the pH range of, Carbopol 934 – 4.5 to 6.0, HPMC K4M – 5.2 to 6.8, HEC 250 HHX – 5.0 to 6.5 which is within the recommended pH range of skin. Hence, the gels do not posses potential to cause skin and scalp irritation. There was no significant change in pH values as a function of time for all formulations.

Viscosity:

Viscosity is an important parameter of gel as it has the direct effect on the mechanical and physical properties such as spreadability, consistency, extrudability, ease of application, washability and release of drugs. Viscosity of all the formulations IGC1 to IGN2P3 and marketed formulation were found to be in the range of 27400cps to 88900 cps and 26400 respectively. Viscosity got decreased on the addition of humectant/ plasticizer causing entanglements of polymer chain. It was observed that medicated gel formulations prepared using Carbopol 934 and HPMC K4M showed higher viscosities than formulations prepared using HEC 250 HHX as compared to the marketed formulation.

Spreadability:

Ease of spreadability is one of the important criteria for a hair gel as there is a need to cover maximum or total area for treatment. Spreadability plays an important role in patient compliance and help in uniform application of gel to the scalp. A good gel take less time to spread and will have high spreadability. Spreadability of gels formulated with HEC 250 HHX as a gelling agent was found to be more than that of formulated with Carbopol 934 and HPMC K4M. Spreadability got increased upon the addition of glycerine and propylene glycol.

Extrudability (Tube Test):

The extrusion of gel from the tube is an important during application and for patient compliance. Gel with high consistency may not extrude form tube easily, whereas low viscous gels may flow quickly. All the formulations showed good to excellent extrudability. Extrudability of the gel formulations with humectant cum plasticizer was excellent and that of formulations without humectant was good.

Drug content :

The % content of Ivermectin of all gel formulations indicated uniform distribution of drug. The drug content of the gel formulations was in the range of 95.52 ± 0.11 to 98.94 ± 0.03, showing content uniformity.

***In vitro* diffusion study –**

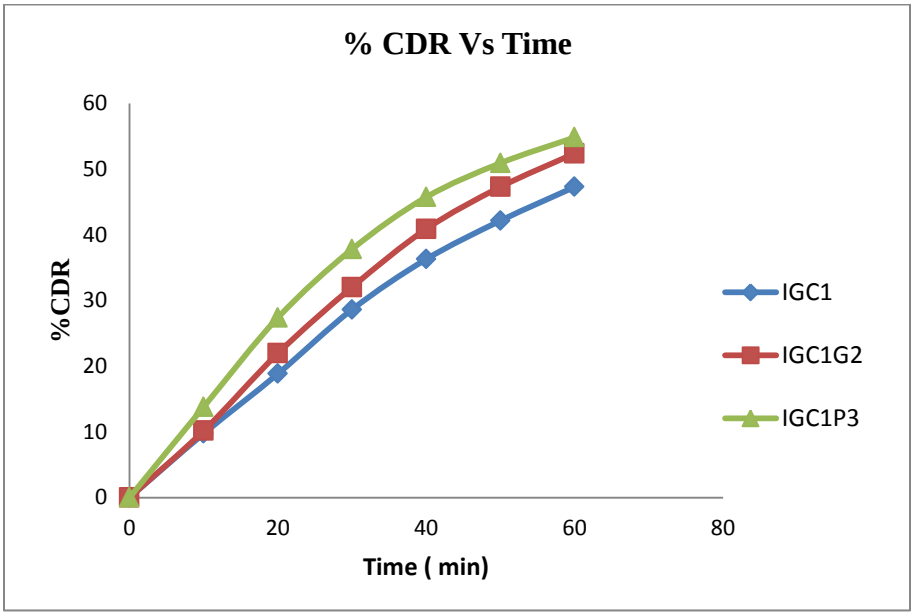

Figure 4: Diffusion (*in vitro*) of ivermectin from gels prepared with carbopol 934

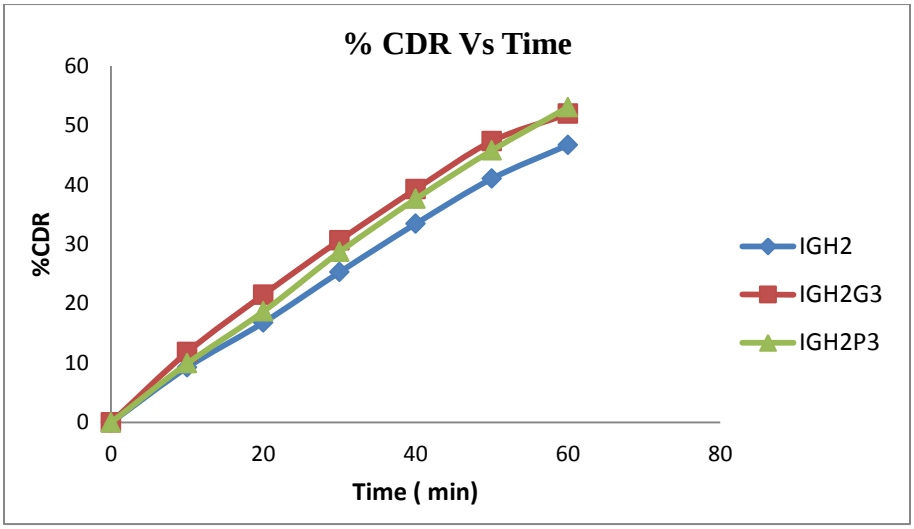

Figure 5: Diffusion (*in vitro*) of ivermectin from gels prepared with HPMC k4m

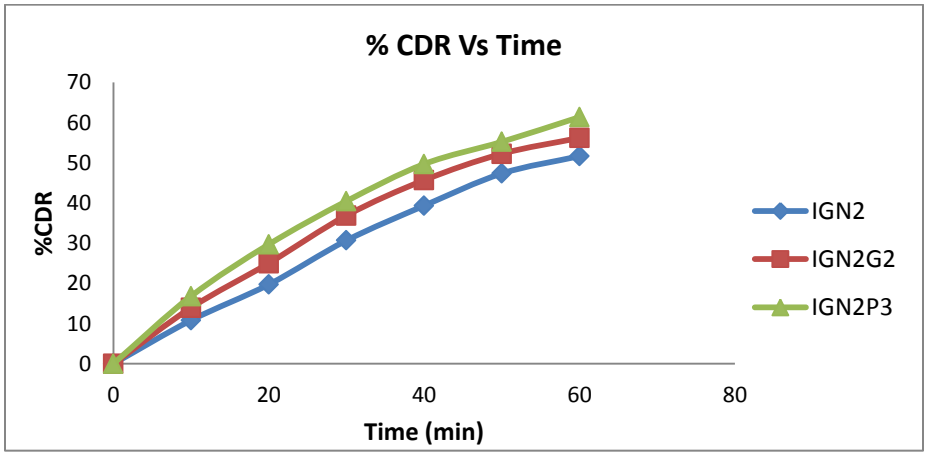

Figure 6: Diffusion (in vitro) of ivermectin from gels prepared with HEC 250 HHX

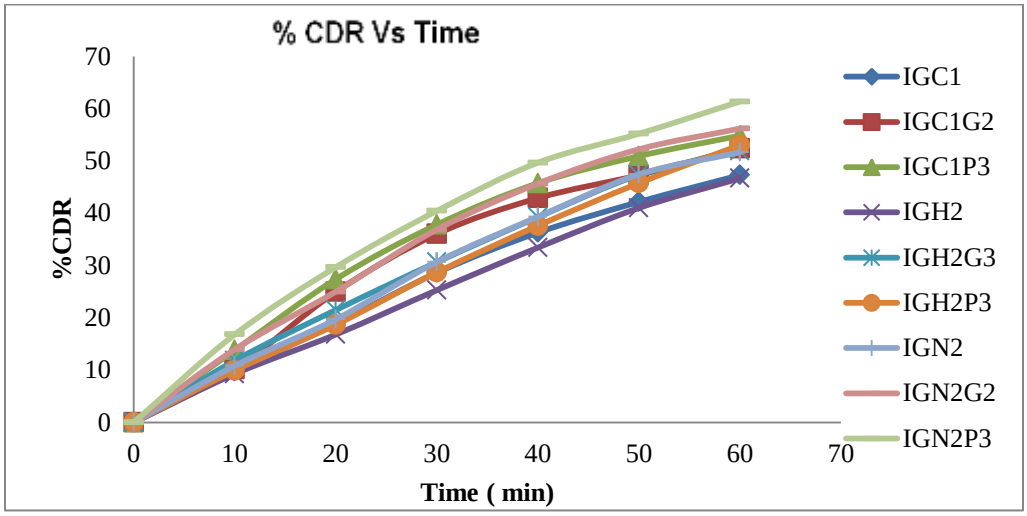

Figure 7: comparative in-vitro drug release profiles of ivermectin gels through synthetic membrane

The diffusion of Ivermecin through gel was in the order of HEC 250 HHX > Carbopol 934 > HPMC K4M. This may be attributed to difference in shape of three dimension structure of the polymer, and shear thinning ability of polymer. The three polymers demonstrated variable capability of polymer network for retention of entrapped Ivermectin thus Carbopol 934 and HPMC K4M possesed greater barrier for diffusion of Ivermectin than HEC 250 HHX. The type of plasticizer and concentration influenced the drug release as plasticizers reduce polymer-polymer chain secondary bonding, and provide more mobility for the drug. Among of them gels of Ivermectin prepared using HEC 250 HHX as gelling polymer and propylene glycol (15%) humectant cum plasticizer facilitated greater diffusion than Carbopol 934, HPMC K4M gels.

Antilice Activity :

The hair gel formulation IGN2P3 was subjected for *in vitro* pediculicidal activity as it exhibited excellent viscosity, spreadability, extrudability and good drug release when compared to other formulations. Hence IGN2P3 was considered as a best formulation. Results of *In vitro* Pediculicidal activities are as shown in table 2.

Table 2: I*n vitro* pediculicidal activity of different groups

Sr. No.	Group	No. of lice released in petriplate	% Mortality Mean	
			5 min	10 min
1	Group I (Control)	10	0	0
2	Group II (Gel base)	10	0	0
3	Group III (Ivermectin gel)	10	50	100
4	Group IV (Marketed gel)	10	40	90

*Each reading is an average of three determinations

*Each reading is an average of three determinations

Data analyzed by ANOVA followed by Tukey Kramer test. Significant difference was found in pediculicidal activity of control vs test formulation (**P < 0.01), gel base vs. test formulation (**P < 0.01). There was not significant difference found in marketed formulation and test formulation (P > 0.05).

The lice were judged as dead if there were no vital signs such as movements of antennae or no leg movements (with or without stimulation by a forceps). All the results were done triplicate. Normal time of head louse to be dead on body of infested person is 1 month and more, but its infestation affects the social life of a person. People tends to avoid come closer to the infested person. Also it gives rise to secondary infections. So, it is important to kill the lice after infestation.

Stability Study:

The hair gel formulation IGN2P3 was subjected to stability performance as it was exhibited good drug release, excellent viscosity, spreadability and pediculicidal activity. The stability study was conducted for the period of 45 days at 25°C/60% RH and 40°C/75% RH. The parameters like appearance, pH, extrudability, colour, % drug content were tested after every 15 days. No appreciable changes were found for the tested parameters. The results of stability are shown in

Skin Irritation test:

The Ivermectin gel formulation IGN2P3 which showed best characteristics and release of Ivermectin was short listed for *in-vivo* animal studies. The animal's skin was observed for toxic manifestation if any after application of formulation at the pre-selected time intervals i.e. 1 hr, 4 hrs, 12 hrs, 24 hrs, 48 hrs, and 72 hrs. The treated animals were apparently free from any irritation symptoms and erythema. This indicates better skin acceptability of topical Ivermectin gel formulation.

CONCLUSION

From the above results it can be concluded that, formulation IGN2P3 containing 2% HEC 250 HHX and 15% propylene glycol was better compared to others. The optimized gel IGN2P3 exhibited the optimal viscosity, spreadability and extrudability to qualify as topical delivery system for local treatment of Pediculosis capitis. It possesed better drug diffusion for a period of 60 min (61.37%). Skin irritation study indicated that no irritation have been produced. It exhibited better pediculicidal efficacy within 10 min than commercially available antilice gel. The Ivermectin gel IGN2P3 was stable and did not show any significant change in viscosity, drug content, *in vitro* drug diffusion studies pattern after stability studies at 25^0C/60% and 40^0C/75% RH for 45 days.

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