ISSN: 2321-3760

Research Article

International Journal of Current Trends in Pharmaceutical Research

IJCTPR, 2014, Vol. 2(3): 459-467

www.pharmaresearchlibrary.com/ijctpr

Formulation and Evaluation of Novel Topical Emulgel of Chlorpheniramine Maleate

Hyma.P[1*], Noor Jahan[2], Raheemunissa[3], Sreelekha G[4], Babu K[5]

Department of Pharmaceutics, Jyothishmathi college of Pharmacy, Hyderabad, India

Received: 27 February 2014, Accepted: 17 April 2014, Published Online: 15 May 2014

Abstract

Chlorpheniramine maleate belongs to the first generation alkylamine antihistaminic classification of histamine antagonists. This is a lipid soluble amine which is useful in the treatment of allergic disorders like conjunctivitis and rhinitis. The objective of the present work was to formulate and evaluate a novel topical emulgel of this antihistaminic drug i.e, Chlorpheniramine maleate. The beneficial effect of antihistaminics in atopic dermatitis is due to their antipruritic and local anaesthetic actions. Chlorpheniramine maleate is usually used, as it was proved to be non interfering with the other normal activities of the body. Carbopol, Hydroxy propyl methyl cellulose are used as polymers or gelling agent, the influence of type and concentration of them on the release of chlorpheniramine was investigated. Tween, span, propylene glycol, oleic acid were used as penetration enhancers, the effect of these on diffusion of chlorpheniramine maleate across the semi permeable membrane was tested. The optimized formulation i.e, F8, which was found to release the maximum dose of the drug, is then tested in vivo on the rat skin and observed for any allergic reactions. Results revealed that the optimized formula containing 2gm HPMC and 1gm propylene glycol was found to relieve allergic reactions compared to commercially available emulgel formulation i.e, Voltaren. So it can be concluded that topical emulgel enhanced permeation of Chlorpheniramine maleate with avoidance of GIT adverse effect.

Keywords: Chlorpheniramine maleate, Carbopol 934, HPMC, Drug content, Skin irritation studies.

Contents

***Corresponding author**
Hyma.P
Jyothishmathi college of Pharmacy,
Hyderabad, India
E-mail: mariyanoor02@gmail.com
Manuscript ID: IJCTPR2053

PAPER-QR CODE

1. Introduction

Chlorpheniramine maleate is an alkyl amine antihistaminic drug which is used to relieve allergy, hay fever, common cold etc. These agents act by either inhibiting H1 receptors or by blocking Acetylcholine receptors which helps to relieve runny nose. The common side effects include drowsiness; dry mouth, nose and throat; nausea; vomiting; loss of appetite; headache; increased chest pain. Severe side effects result in vision problems, difficulty in urinating, etc [(1)]. Chlorpheniramine maleate is rapidly and almost completely absorbed from the GI tract, so this is given orally 3-4 times a day. Hence in order to avoid this patient incompliance this is formulated into topical preparations such as gels, emulgels, creams etc [2].

Ophthalmic, rectal, vaginal and skin are the topical routes for localized drug delivery in the body. Skin is regarded as one of the most readily accessible organs on human body for topical administration [4]. Topical products applied to skin differs in their formulation and range in their consistency from liquid to powder, of these the most popular products are semisolid preparation.Within this major group of semisolid preparations, the transparent gels have been used expandedly both in cosmetics and in pharmaceutical preparations. These formulations can also be used to manipulate the barrier function of the skin, Stratum corneum, which presents more than 99% of the total skin surface for percutaneous absorption[6]. In order to enhance the percutaneous absorption various agents such as permeation enhancer like propylene glycol is added to the formulation.

Emulsion is a biphasic system in which one immiscible liquid is dispersed into other, but due to its instability emulsifying agents are added. Emulsion is used as vehicle to deliver drug. They can be easily washed off from skin and have good penetration capability. The dosage form created by entrapment of large amounts of aqueous or hydroalcoholic liquid in a network of colloidal solid particles are called as gels. These consist of inorganic substances like aluminum salts or organic polymers of natural or synthetic origin [8]. Emulgel, dermatologically, has several favorable properties such as being thixotropic, greaseless, easily spreadable, easily removable, emollient, non-staining, and transparent with long shelf life and pleasing appearance[9].Other advantages include; better stability, high loading efficiency, high production and economical with low cost. The ideal emulgel is prepared by mixing an emulsion either water-in-oil (W/O) type or O/W with a gelling agent. The drug is practically insoluble in water, soluble in lipid amines [10]. The aim of this work is to formulate chlorpheniramine maleate as emulgel, so as to eliminate the GIT side effects, to incorporate this insoluble drug in a hydrophilic gel matrix, and to enhance the percutaneous absorption and pharmacodynamic of this drug [11].

2. Materials and Methods

Materials:

Chlorpheniramine maleate was purchased from Yarrow chem. Products, Mumbai. Hydroxy propyl methyl cellulose, Carbopol, Span 80, tween 80, Oleic acid, Acetone, Propylene glycol, Light liquid paraffin, preservatives such as Methyl paraben and propyl paraben were purchased from Sd-Fine Chemicals limited, Mumbai.

Methodology:

Preparation of Chlorpheniramine maleate emulgel:

The preparation of emulsion was same in all the formulations. Different formulations of the drug were prepared by using varying types and concentration of gelling agents or polymers such as carbopol, HPMC.

Aqueous phase:

The aqueous phase was prepared by dissolving drug in sufficient quantity of water. Tween 80 was added to this dissolved drug mixture. Dissolve propylene glycol in specified quantity of methyl paraben and propyl paraben. Then add this mixture to the aqueous phase which was prepared earlier.

Oil phase:

According to the mentioned formula dissolve Span 80 in Light liquid paraffin. Then add oleic acid which was screened as oil phase.

Emulsion:

Both oil phase and aqueous phase were heated separately at 70^0 c using the heating mantles. Then the oil phase was added to the aqueous phase, while hot, with continuous trituration resulting in an optimized emulsion.

Gel: Gel bases were prepared by using varying amounts of gelling agents such as Carbopol and HPMC. The polymer was dissolved in sufficient quantity of distilled water. This mixture was heated at 75^0c with continuous stirring until a transparent, viscous and stable gel was formed.

Emulgel:

1gm of gel base was accurately weighed and taken in a mortar. Then the previously prepared emulsion was added dropwise to the gel base in 1:1 weight ratio with continous trituration. This results in the required emulgel formula. Various proportions of the polymers such as carbopol and HPMC were used to prepare other formulations of Chlorpheniramine maleate according to the procedure mentioned above.

Preparation of HPMC Emulgel:

The gel bases (0.5, 1, 1.5, 2) were prepared by dissolving HPMC K_4M in sufficient quantity of water while heating at 75°c. This suspension was cooled and left overnight. The drug emulsion was prepared according to the given formula. This prepared emulsion was added to the HPMC gel bases in 1:1 weight ratio with continuous trituration, resulting in a appropriate emulgel.

Preparation of Carbopol Emulgel:

The gel bases (0.5, 1, 1.5, 2) were prepared by dissolving Carbopol in sufficient quantity of water while heating at 75°c. This suspension was cooled and left overnight. The drug emulsion was prepared according to the given formula. This prepared emulsion was added to the Carbopol gel bases in 1:1 weight ratio with continuous trituration, resulting in a transparent emulgel.

Table 1. Formulation of chlorpheniramine maleate

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Drug	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Span 80	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Oleic acid	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Light liquid paraffin	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Acetone	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Propylene glycol	1	1	1	1	1	1	1	1
Methyl paraben	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
Propyl paraben	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
Carbopol	0.5	1	1.5	2	-	-	-	-
HPMC	-	-	-	-	0.5	1	1.5	2
Water	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

Evaluation and Characterization:

Physical Appearance:

The prepared Chlorpheniramine maleate emulgel formulations were inspected visually for their pH, colour, homogeneity, consistency, grittiness and phase separation [(12)].

pH Measurement:

The pH of various emulgel formulations was determined with the help of digital pH meter.1 gram of gel was dissolved in 100 ml of distilled water and it was left aside for 2 hours. The pH of each formulation was measured and was done in triplicate and average values were calculated. Based on the obtained values the optimized formula was selected [(13)].

Spreading Coefficient:

Spreadability was determined by apparatus suggested by Mutimer et al (1956). It consists of a wooden block, which is provided by a pulley at one end. By this method, spreadability was measured on the basis of 'Slip' and 'Drag' characteristics of emulgels. A ground glass slide is fixed on this block. An excess of emulgel (about 2 gm) under study is placed on this ground slide. The emulgel 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 weight was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the emulgel between the slides. Excess of the emulgel was scrapped off from the edges. The top plate was then subjected to pull of 80 gms. 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 spread ability.

Extrudability Study of Topical Emulgel (Tube Test):

It is a usual empirical test to measure the force required to extrude the material from tube. The method applied for determination of applied shear in the region of the rheogram corresponding to a shear rate exceeding the yield value and exhibiting consequent plug flow.In the present study, the method adopted for evaluating emulgel formulation for extrudability is based upon the quantity in percentage of emulgel and emulgel extruded from lacquered aluminum collapsible tube on application of weight in grams required to extrude at least 0.5 cm ribbon of emulgel in 10 seconds. More quantity extruded, better is extrudability. The measurement of extrudability of each formulation is in triplicate and the average values are presented. The extrudability is then calculated by using the following formula:

Extrudability = Applied weight to extrude emulgel from tube (in gm) / Area (in cm2) [(14)]

Drug Content Determination: 1g of the prepared chlorpheniramine maleate emulgel was mixed with 100 ml of suitable solvent (methanol). Aliquots of different concentration were prepared by suitable dilution after sonication. Then filter the stock solution and absorbance was measured at 267 nm by using UV-Visible spectrophotometer. Drug content was calculated [(15)].

In Vitro Diffusion Studies:
The diffusion studies of the prepared emulgels can be performed using Franz diffusion cell for studying the dissolution release of emulgels through a cellophane membrane. Gel sample (1g) is taken in cellophane membrane and the diffusion studies were carried out at 37 ± 1° using phosphate buffer (pH 6.4) as the dissolution medium. 5 ml of each sample is withdrawn periodically at 1, 2, 3, 4, 5and 6 hrs and each sample is replaced with equal volume of fresh buffer solution. Then the samples were analyzed for the drug content by using phosphate buffer as blank at 267 nm by using UV-Visible Spectrophotometer [(16)].

Ex Vivo Diffusion Studies:
Ex vivo diffusion study was carried out by using rat skin, and procedure was similar to that of in vitro diffusion study. Cumulative corrections were made to obtain the total amount of drug diffused at each time interval and ex vivo parameters were calculated. The average cumulative amount of drug permeated per unit surface area of the skin was plotted versus time. The slope of the linear portion of the plot was calculated as flux J_{ss} **(**μg /cm2/h), and the permeability coefficient was calculated using the following formula:

$$\mathbf{K_P = J_{SS}/C_U}$$

Where; K_P is the permeability coefficient and C_U is the total amount of drug.
The enhancement of drug penetration due to emulsion formulation compared with marketed emulgel Voltarol was noted as enhancement factor (EF) which was calculated using the following formula:

$$EF = K_P \text{ (emulsion based gel)} / K_P \text{ (Chlorpheniramine maleate)}.$$

Skin Irritation Study:
The emulgel preparation was applied on the properly shaven skin of male wister rat and changes in color, change in skin morphology should be checked up to 24 hours. A set of 3 rats was used for the study. If no irritation occurs the test is passed. If the skin irritation symptom occurs in more than 2 rats the study should be repeated [(17)].
Ftir Spectral Studies:
The IR absorption spectrum of the pure drug, chlorpheniramine maleate, and drug along with the excipients used were taken in the range of 4000-400 cm-1 using KBr pellet method.

3. Results and Discussion

Physical Appearance:
All the prepared emulgel formulations were white, viscous creamy preparation with a smooth homogeneous texture and glossy appearance.

Table 2. Physicochemical characteristics of formulations

Formulation	Colour	Phase separation	Grittiness	Homogeneity	Consistency
F 1	White	None	-	Good	Good
F2	White	None	-	Good	Satisfactory
F3	White	None	-	Excellent	Good
F4	White	None	-	Fair	Good
F5	White	None	-	Good	Satisfactory
F6	Transparent	None	-	Good	Excellent
F7	Transparent	None	-	Excellent	Excellent
F8	Transparent	None	-	Excellent	Excellent

P^H Measurement:
The Ph of the emulgel formulations was found to be in the range of 5.5 to 6.4, which lies in the normal pH range of the skin and will not give any skin irritation.
Spreading Coefficient:
The spreadability indicates the ease with which the emulgel spreads by small amount of shear. The spreading coefficient values lie in the range of 25 to 38 indicating good spreadability of the gels.
Extrudability:
The chlorpheniramine maleate formulations were found to have optimum extrusion characteristics as they were found to extrude out of the tubes with minimum force as observed with the test been done on all the formulations.
Drug Content:
In all the formulations of chlorpheniramine maleate the drug content was found in the range of 86.12 to 98.26 as shown in the following table.

Table 3. Drug content of the formulations

Formulations	Drug Content
F 1	88.25
F2	86.12
F3	90.23
F4	86.24
F5	87.12
F6	96.12
F7	97.25
F8	98.26

In Vitro Drug Release Studies:

The release of chlorpheniramine from the emulgel was found to vary according to concentration of polymer. The release of the drug was found to increase as the formulations were varying with different polymers and different strengths. The release rate can be attributed to effective drug diffusion through the gel phase across the membrane.

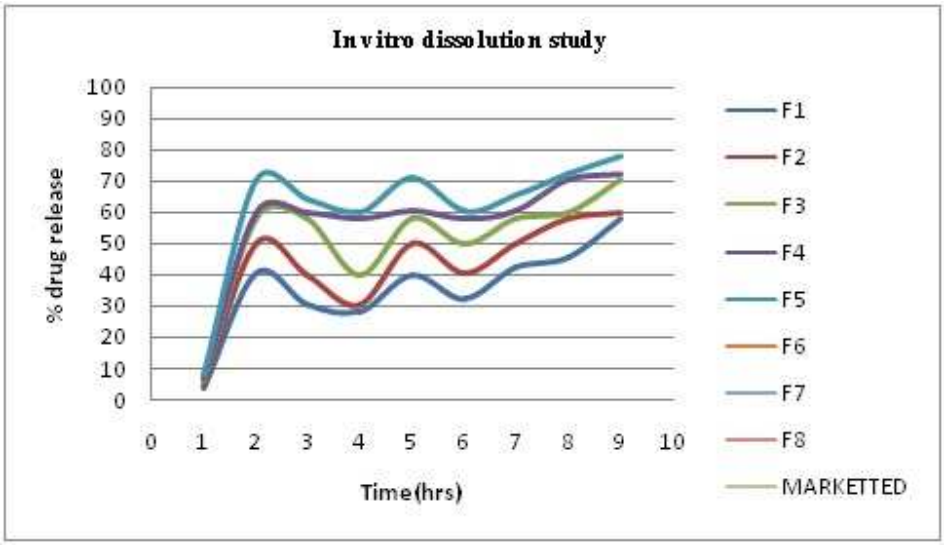

Figure 1. In vitro drug release data

Ex Vivo Drug Release Studies: The study was conducted and the optimized formulation was found to correlate with the values of in vitro drug release studies.

FTIR Spectral Studies:

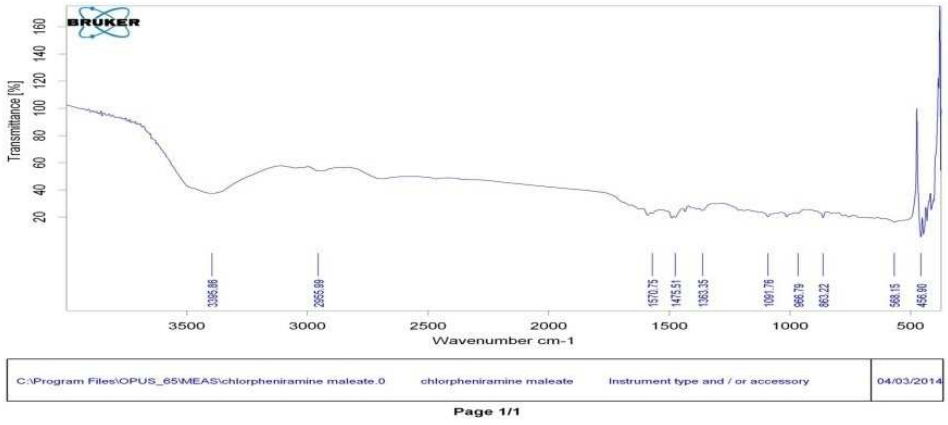

Figure 2. FTIR Spectra of Chlorpheniramine Maleate

Table4. FTIR spectra of chlorpheniramine maleate shows characteristic peaks in the following range

Range	Nature of bond
1580 cm^{-1}	C=C stretching
1475 cm^{-1}	C=H stretching
1352 cm^{-1}	C-H stretching
863 cm^{-1}	C-C stretching
702 cm^{-1}	C-Cl stretching

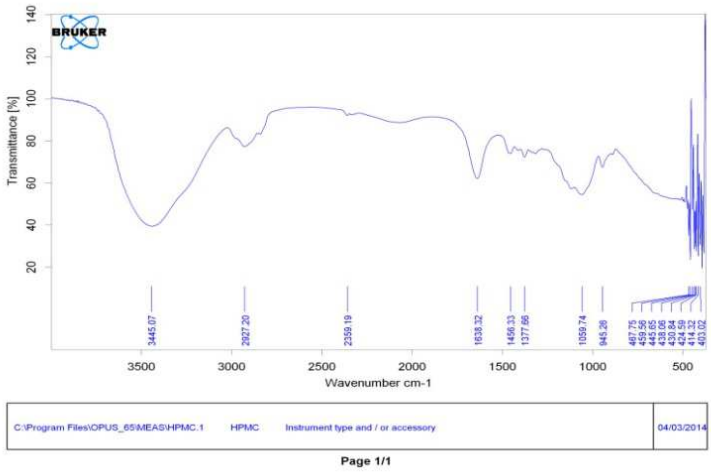

Figure 3. FTIR Spectra of HPMC

Table 5. FTIR spectra of HPMC polymer shows characteristic peaks in the following range

Range	Nature of bond
3445 cm^{-1}	O-H stretching
945 cm^{-1}	C-O stretching
1059 cm^{-1}	C-O stretching
3445 cm^{-1}	OH stretching

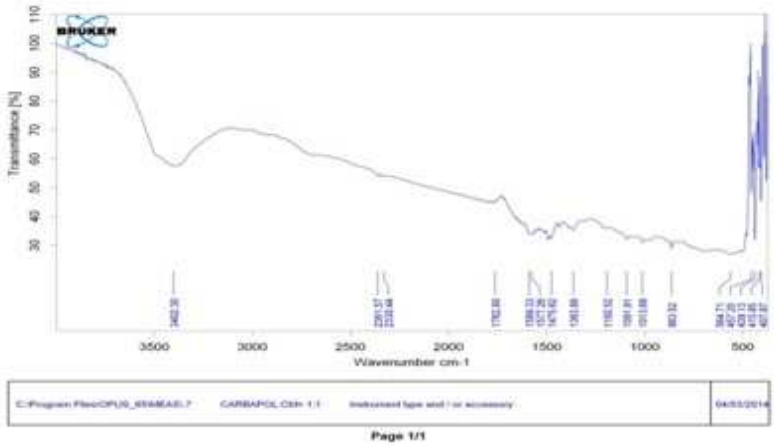

Figure 4. FTIR Spectra of Carbopol

Table 6. FTIR spectra of CARBOPOL polymer shows characteristic peaks in the following range

Range	Nature of bond
3447 cm^{-1}	O-H stretching
2926 cm^{-1}	O-H stretching
1171 cm^{-1}	C-O-C stretching

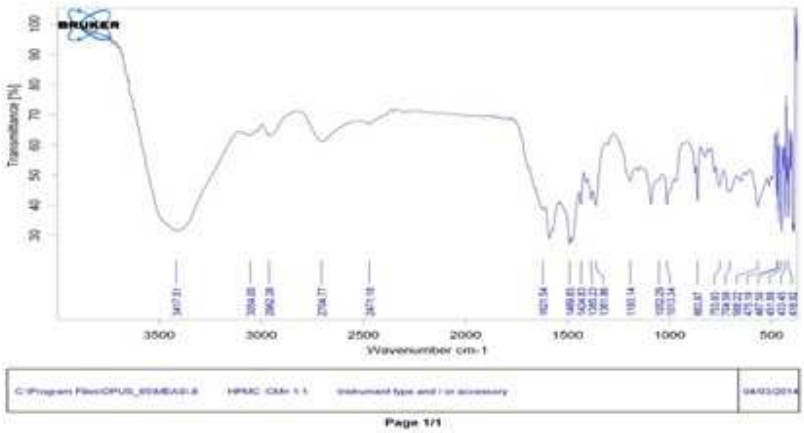

Figure 5. FTIR Spectra of the Drug and the Polymer

In the FTIR spectra of drug combined with polymers the same peaks can be observed of the drug and polymer with no considerable shift in the peaks, suggesting that there was no interaction of the drug with the polymers used in the formulation.

Skin Irritation Study: The primary irritation index of the sample was observed to be zero. Hence no irritation was observed on the skin of the rabbit i.e, no allergic symptoms like inflammation, redness, irritation appeared on the rats upto 24 hrs.

Effect of vaccination on HI titer

Average hemagglutination inhibition (HI) antibody titer of sera after parvovirus vaccination of pups in different treatment groups have been presented in Table 1. On 0 day the critical difference test showed there was no significant difference between the different groups. On 28th day the highest titer was observed in the pups treated with Megavac 6 which showed a significant difference ($P<0.01$) with the control group. Rest of the group did not show significant difference among themselves. On 35th day the lowest antibody titer was observed in Group 4.

Effect of vaccination on serum neutralization antibody titer

On the 28th day, group 1 showed a highest antibody. Group 1 showed significant difference ($P<0.01$) between the treatment groups. On the 35th day, the highest antibody titer was observed in group 1 and the lowest titer in Group 4 (Table 2). Common problem in vaccinating canines, such as dogs and puppies, is the maternal antibody interference during immunization. Maternal antibody interference is the most common cause of vaccine failure in weaning pups. Maternal antibody neutralizes vaccine and suppresses pup´s active immune response. This common immunization problem occurs with all disease, but is of particular concern in the case of CPV´s enteritis because of the explosive nature of disease transmission and because pups are at greatest risk of CPV-induced mortality [5]. Tizard [6] reported a significant increase in antibody titer on 3 wks of post first vaccination. After 3 weeks of 2nd vaccination, it was observed that a significant decrease in HI antibody titer happened, but was above protective titer of 1:80. In the control group there was no significant variation between the different days. He concluded from his study that vaccination with Vanguard which contains a CPV2 strain help to protect dog against a virulent infection with CPV 2c-type.

Bergman et al. [7] reported that the puppies vaccinated with commercially available modified live vaccine against CPV, provided adequate protection with final vaccination at 10 weeks age.

Reddy et al. [8] vaccinated the pups with Megavac 6 and collected serum sample on 720 day post vaccination to assess the serum antibody response and observed the HI titer 600.

Wanner et al. [5] vaccinated pups with modified attenuated CPV vaccine at 6 and 9 wks of age observed 39% of the pups responded to vaccination at the HI titer is 1:10, 30% of pups at 1:20, 26% at 1:40 and 5% at 1:80.

Table 7. HI antibody titer (log_{10} values) of pup sera

Day	Group 1	Group 2	Group 3	Group 4 (Control)
0 day	1.95±0.21	1.65±0.25	1.60±0.25	2.20±0.11
28th day	2.91±0.23^{b}	2.66±0.29b^{c}	2.40±0.27^{bc}	1.70±0.15^{a}
35th day	3.86±0.15^{b}	2.71±0.17^{c}	2.61±0.19^{c}	1.35±0.05^{a}

Values bearing same superscript in a row did not differ significantly. All the values were expressed in Mean ± S.E, ($P<0.01$)

Table 8. SNT antibody titer (log_{10} values) of pup sera between groups

Day	Group 1	Group 2	Group 3	Group 4 (Control)
0-Day	2.00±0.15	1.60±0.19	1.70±0.13	1.75±0.10
28-Day	$2.81±0.25^{c}$	$2.30±0.13^{b}$	$1.95±0.16^{ab}$	$1.60±0.11^{a}$
35-Day	$3.36±0.12^{c}$	$2.40±0.24^{b}$	$2.30±0.15^{b}$	$1.45±0.07^{a}$

Values containing same superscript in row did not differ significantly. All the values were expressed in Mean ± S.E, ($P<0.01$)

In the FTIR spectra of drug combined with polymers the same peaks can be observed of the drug and polymer with no considerable shift in the peaks, suggesting that there was no interaction of the drug with the polymers used in the formulation.

Skin Irritation Study:

The primary irritation index of the sample was observed to be zero. Hence no irritation was observed on the skin of the rabbit i.e, no allergic symptoms like inflammation, redness, irritation appeared on the rats upto 24 hrs

4. Conclusion

The present study reports for the development chlorpheniramine maleate emulgel for topical release of the drug. On the basis of the above findings the following could be concluded that the nature of the polymers used in preparation of gels and their concentrations showed an effect on release of chlorpheniramine maleate from emulgel base. The maximal enhancement of chlorpheniramine maleate permeation was obtained with formula F8 which showed maximum cumulative amount permeated when observed for 8hrs. It can be conclusively stated that the chlorpheniramine maleate emulgel formulation appears to be the promising system for the topical delivery of chlorpheniramine maleate to avoid the disturbances of the conventional routes of administration. Nevertheless, more systematic research should be conducted to prove the feasibility of emulgel delivery systems for parenteral administration.

5. References

1. Hasenöhrl, R. U.; Weth, K.; Huston, J. P. "Intraventricular infusion of the histamine H1 receptor antagonist chlorpheniramine improves maze performance and has anxiolytic-like effects in aged hybrid Fischer 344×Brown Norway rats". **1999,** 128 (4): 435–440.
2. Fawzia Habib, Maha Abdel Azeem, Amal El Sayeh Fadl And Raafat El Sayed. Formulation Of Ketorolac Tromethamine In Semi-Solid Dosage Forms. Bull. Pharm. Sci., Assiut University. December **2009**, 32(2): 257-271.
3. Ranga Priya M, Sellakumar V, Natarajan R And Mohan Kumar K. Formulation And In-Vitro Evaluation Of Ciprofloxacin Loaded Topical Emulgel. International Journal Of Pharmaceutical And Chemical Sciences. Jan – Mar **2012**, 1 (1), 237-242.
4. Joshi Baibhav, Singh Gurpreet 1, Rana AC2, Saini Seema1. Development and Characterization of Clarithromycin Emulgel for topical delivery. International Journal of Drug Development & Research 4 (3); July-September **2012,** 310-323.
5. Dadwal Meenakshi. Emulgel: A Novel Approach To Topical Drug Delivery. Int J Pharm Bio Sci 4(1); Jan 2013: 847–856.
6. Joshi Bhaibhav, Singh Gurpreet, Rana A.C2, Saini Seema, Singla Vikas. Emulgel:A comprehensive review on the recent advances in topical drug delivery. Int. Research Journal of Pharmacy. **2012,** 2(11): 66-70.
7. S. B. Kute and R.B. Saudagar1. Emulsified gel A Novel approach for delivery of hydrophobic drugs: An overview. J. Adv. Pharm. Edu. & Res. Vol. 3 Issue 4, Oct-Dec **2013**, 368-376.
8. Rachit Khullar, Deepinder Kumar, Nimrata Seth, Seema Saini. Formulation and evaluation of mefenamic acid emulgel for topical delivery. Saudi Pharmaceutical Journal, 2012, 20: 63–67.
9. Dignesh M. Khunt, Ashish D. Mishra, Dinesh R. Shah. Formulation Design & Development of Piroxicam Emulgel. International Journal of Pharm Tech Research. 4(3), July-Sept **2012**, 1332-1344.
10. Olga Popovska, Vesna Rafajlovska, Zoran Kavrakovski. A Review: Current Analytical Methods for Determination of Ketoconazole in Pharmaceutical and Biological Samples. IJPI's Journal of Analytical Chemistry. **2013**, 3(13): 1-13.
11. Monica Rao, Girish Sukre, Sheetal Aghav, and Manmeet Kumar. Optimization of Metronidazole Emulgel. Journal of Pharmaceutics.): **2013,** Volume 20(3), 9-16.

12. Khaled M. Hosny, Shatha M. Rambo3, Muna M. Al-Zahrani3, Samia M. Al-Subhi3, Usama A. Fahmy1. Ketoprofen Emulgel: Preparation, Characterization, and Pharmacodynamic Evaluation. Int. J. Pharm. Sci. Rev. Res., May – Jun **2013**, 20(2): 306-310.
13. Nahla Salah Barakat . Evaluation of Glycofurol-Based Gel as a New Vehicle for Topical Application of Naproxen. AAPS PharmSciTech. **2010** September; 11(3): 1138–1146.
14. Sonaje Sumeet Purushottam, Gondkar Sheetal Bhaskarrao2, Saudagar Ravindra Bhanudas3. Gellified Emulsion: A New Born Formulation for Topical Delivery of Hydrophobic Drugs. World journal of pharmacy and pharmaceutical sciences. 3(1): 233-251.
15. S J Shelke, DM. Shinkar, RB. Soudagar. Topical gel: A novel approach for development of topical DDS, Int.J. of Pharmacy and technology, **2013**. 3(3), 480-490.
16. Vikas Singla, Seema Saini1, Baibhav Joshi1 and A.C Rana2. Emulgel: A New Platform for Topical Drug Delivery. International Journal of Pharma And Bio Sciences. Jan – Mar **2012**, 3(1), 485-498.
17. Dadwal Meenakshi. Emulgel : A Novel Approach To Topical Drug Delivery. Int J Pharm Bio Sci., **2013** Jan; 4(1): (P) 847 – 856.