Research Article

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Formulation and ex -vivo evalaution of chlorzoxazone transdermal emulgel by skin irritation test

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ABSTRACT

Emulgel is one of the recent technologies in NDDS used for dual control release of emulsion and gel for topical use. Gel formulations generally provide faster drug release compared with conventional ointments and creams. Chlorzoxazone is a well-known therapeutic agent that is used mainly for its skeletal muscle relaxants. The aim and objective of the study is to formulate Chlorzoxazone emulgel for topical application. Emulgel of Chlorzoxazone, consist of Carbopol-940 or HPMCk4 as a gelling agents for gel formulation and tween 80,span 20, for emulsion formulation. Emulgel was formulated by emulsion incorporated in gel. Chlorzoxazone loaded emulgel was formulated by using o/w emulsion because of lower solubility in water. Lemon grass oil, Menthol was used as a penetration enhancer in emulgel formulation. Optimized formulation was evaluated for physical examination, swelling index, skin irritation study, extrudability study, drug content determination, spreadability, globule size determination and in-vivo drug release, rheological study. Optimized formulation gave drug release 98.8% for 12 hrs. The selected gel formulations were found to show no redness of skin and no skin irritation. The formulation was found to be safe when topically applied.

Keywords: Emulgel, Chlorzoxazone, Spreability, Irritancy Test.

INTRODUCTION

The delivery of drugs across the skin is gaining wide acceptance among patients and termed as Topical drug delivery. It is a viable administration route for potent, low molecular weight therapeutic agents susceptible to first pass metabolism. Topical drug delivery is referred to as a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal and skin as topical routes. Skin is one of the most readily accessible organs on human body for topical administration and is main route of topical drug delivery system.

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In developing a transdermal delivery system, two criteria are considered: one is achieving adequate flux across the skin and the other is minimizing the lag time in skin permeation [1].

Physiology of Skin

Most of the topical preparations are meant to be applied to the skin. So, basic knowledge of the skin and its physiology function are very important for designing topical drug delivery. The skin of an average adult body covers a surface area approximately 2m2 and receives about one third of the blood circulating through the body. An average human skin surface is known to contain, the average 40-70 hair follicles and 200-300 sweat ducts on every square centimeter of the skin. The pH of the skin varies from 4 to 5.6. Sweat and fatty acid secreted from sebum influence the pH of the skin surface. The skin can be considered to have four distinct layers of tissue as shown [2] in Figure 1.

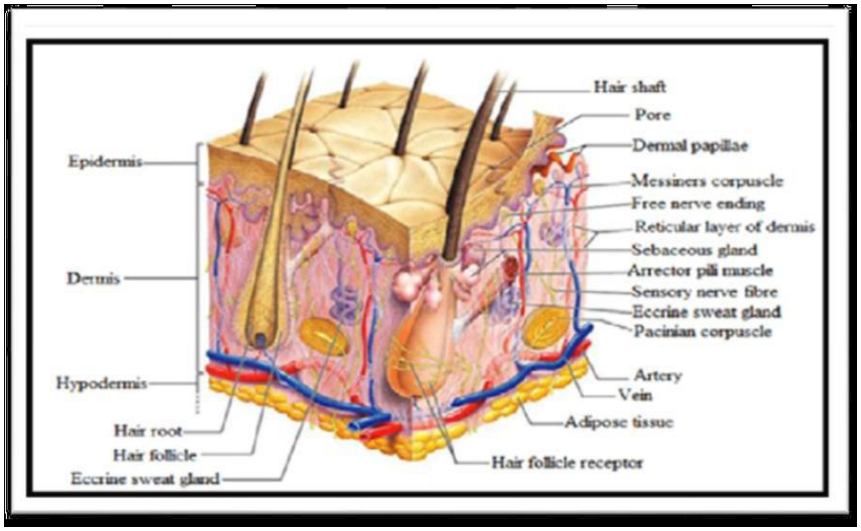


Fig 1: Physiology of skin

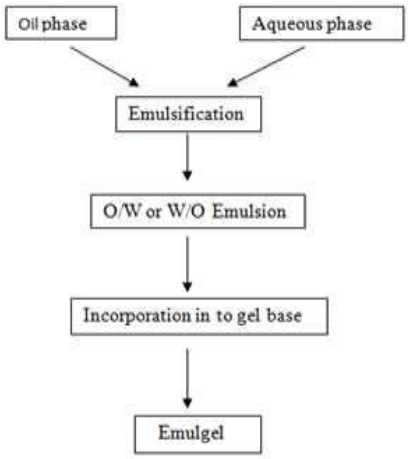
Drug Delivery across the Skin

A unique aspect of dermatological pharmacology is the direct accessibility of the skin as a target organ for diagnosis and treatment. The skin acts as a two-way barrier to prevent absorption or loss of water and electrolytes. There are three primary mechanisms of topical drug absorption: transcellular, intercellular, and follicular. Most drugs pass through the torturous path around corneocytes and through the lipid bilayer to viable layers of the skin. The next most common (and potentially under recognized in the clinical setting) route of delivery is via the pilosebaceous route. The barrier resides in the outermost layer of the epidermis, the stratum corneum, as evidenced by approximately equal rates of penetration of chemicals through isolated stratum corneum or whole skin [3].

Materials

Chlorzoxazone,Lemon grass oil was purchased from Yarrow chemicals (Mumbai), Carbopol 940,HPMCk4,Menthol,Arachis oil,Cetostearyl Alcohol,Propylene Glycol, Tween 80, Span 20, Lemon grass oil, Methyl paraben, Propyl paraben, were purchased from S.D Fine Chemicals Ltd., Mumbai (India) All other chemicals and reagents used were of analytical grade.Deionized distilled water was used throughout the study.

Method



Formulation and development of emulgel formulation

PROCEDURE

Preparation of Emulsion

The emulsion itself consists of oily base and aqueous base. The drug was dissolved in Arachis oil, Span20, Lemon grass oil and cetostearyl alcohol. Then oil phase was prepared.

At the same time the propylene glycol, Tween80, Methyl paraben and Propyl paraben were added in water.Then Aqueous phase was prepared.

Then the oil base was added to aqueous base with continuous stirring at 70^{o}C.

Preparation of gel base

Appropriately weighed amount of viscosity increasing agents dispersed and hydrated in 70% of medium (water + NMP) for 24 H and pH was adjusted to 6-7.4 by using tri ethanolamine.

Mixing of emulsion with gel base

Then mix the both emulsion and gelbase at moderate speed with a mechanical stirrer its form emulge [4].

Table 1: Composition of Formulation (g)

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Chlorzoxazone	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Carbopol 940	0.5	1	1.5	2	2.5	3.0	-	-	-	-		
HPMC K4	-	-	-	-			2	2.5	3	3.5	4.0	4.5
Menthol	1	1	1	1	1	1	1	1	1	1	1	1
Arachis oil	5	5	5	5	5	5	5	5	5	5	5	5
Cetostearyl Alcohol	2	2	2	2	2	2	2	2	2	2	2	2
Propylene Glycol	3	3	3	3	3	3	3	3	3	3	3	3
Tween20	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Span20	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0./5	0.5
Lemon Grass Oil	3	3	3	3	3	3	3	3	3	3	3	3
Methyl Paraben	q.s.	q.s.	q.s.	q.s.	q.s	q.s	q.s.	q.s.	q.s.	q.s.	q.s	q.s
Propyl Paraben	q.s.	q.s.	q.s.	q.s.	q.s	q.s	q.s.	q.s.	q.s.	q.s.	q.s	q.s
Distilled Water	q.s.	q.s.	q.s.	q.s.	q.s	q.s	q.s.	q.s.	q.s.	q.s.	q.s	q.s

SKIN IRRITATION TEST

Test Procedure

Application of the test chemical

The test chemical should be applied to a small area (approximately 6 cm2) of skin and covered with a gauze patch, which is held in place with non-irritating tape. In cases in which direct application is not possible (e.g., liquids or some pastes), the test chemical should first be applied to the gauze patch, which is then applied to the skin. The patch should be loosely held in contact with the skin by means of a suitable semi-occlusive dressing for the duration of the exposure period. If the test chemical is applied to the patch, it should be attached to the skin in such a manner that there is good contact and uniform distribution of the test chemical on the skin. Access by the animal to the patch and ingestion or inhalation of the test chemical should be prevented[6].

Liquid test chemicals are generally used undiluted. When testing solids (which may be pulverized, if considered necessary), the test chemical should be moistened with the smallest amount of water (or, where necessary, of another suitable vehicle) sufficient to ensure good skin contact. When vehicles other than water are used, the potential influence of the vehicle on irritation of the skin by the test chemical should be minimal, if any.

Dose level

A dose of 0.5 mL of liquid or 0.5 g of solid or paste is applied to the test site.

Initial test (*In vivo* dermal irritation/corrosion test using one animal)

When a test chemical has been judged to be corrosive, irritant or non-classified on the basis of a weight of evidence analyses or of previous *in vitro* testing, further *in vivo* testing is normally not necessary. However, in the cases where additional data are felt warranted, the *in vivo* test is performed initially using one animal and applying the following approach. Up to three test patches are applied sequentially to the animal. The first patch is removed after three minutes. If no serious skin reaction is observed, a second patch is applied at a different site and removed after one hour. If the observations at this stage indicate that exposure can humanely be allowed to extend to four hours, a third patch is applied and removed after four hours, and the response is graded [7].

Confirmatory test (*In vivo* dermal irritation test with additional animals)

If a corrosive effect is not observed in the initial test, the irritant or negative response should be confirmed using up to two additional animals, each with one patch, for an exposure period of four hours. If an irritant effect is observed in the initial test, the confirmatory test may be conducted in a sequential manner, or by exposing two additional animals simultaneously. In the exceptional case, in which the initial test is not conducted, two or three animals may be treated with a single patch, which is removed after four hours. When two animals are used, if both exhibit the same response, no further testing is needed. Otherwise, the third animal is also tested. Equivocal responses may need to be evaluated using additional animals.

Observation period

The duration of the observation period should be sufficient to evaluate fully the reversibility of the effects observed. However, the experiment should be terminated at any time that the animal shows continuing signs of severe pain or distress. To determine the reversibility of effects, the animals should be observed up to 14 days after removal of the patches. If reversibility is seen before 14 days, the experiment should be terminated at that time.

All animals should be examined for signs of erythema and oedema, and the responses scored at 60 minutes, and then at 24, 48 and 72 hours after patch removal. For the initial test in one animal, the test site is also examined immediately after the patch has been removed. Dermal reactions are graded and recorded according to the grades in the

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Table below.If there is damage to skin which cannot be identified as irritation or corrosion at 72 hours, observations may be needed until day 14 to determine the reversibility of the effects.In addition to the observation of irritation, all local toxic effects, such as defatting of the skin, and any systemic adverse effects (e.g., effects on clinical signs of toxicity and body weight), should be fully described and recorded.Histopathological examination should be considered to clarify equivocal responses.

The grading of skin responses is necessarily subjective. To promote harmonisation in grading of skin response and to assist testing laboratories and those involved in making and interpreting the observations, the personnel performing the observations need to be adequately trained in the scoring system used (see Table below). An illustrated guide for grading skin irritation and other lesions could be helpful.

Data and reporting

Study results should be summarised in tabular form in the final test report and should cover.

Evaluation of results

The dermal irritation scores should be evaluated in conjunction with the nature and severity of lesions, and their reversibility or lack of reversibility. The individual scores do not represent an absolute standard for the irritant properties of a material, as other effects of the test material are also evaluated. Instead, individual scores should be viewed as reference values, which need to be evaluated in combination with all other observations from the study.

Reversibility of dermal lesions should be considered in evaluating irritant responses. When responses such as alopecia (limited area), hyperkeratosis, hyperplasia and scaling, persist to the end of the 14-day observation period, the test chemical should be considered an irritant.

Erythema and Eschar Formation

No erythema 0
Very slight erythema (barely perceptible)..... 1
Well defined erythema..... 2
Moderate to severe erythema..... 3
Severe erythema (beef redness) to eschar formation preventing grading of erythema 4
Maximum possible: 4

Oedema Formation

No oedema 0
Very slight oedema (barely perceptible) 1
Slight oedema (edges of area well defined by definite raising)..... 2
Moderate oedema (raised approximately 1 mm)..... 3
Severe oedema (raised more than 1 mm and extending beyond area of exposure) 4
Maximum possible: 4

ANNEX DEFINITIONS

Dermal irritation is the production of reversible damage of the skin following the application of a test chemical for up to 4 hours.

Dermal corrosion is the production of irreversible damage of the skin; namely, visible necrosis through the epidermis and into the dermis, following the application of a test chemical for up to four hours. Corrosive reactions are typified by ulcers, bleeding, bloody scabs, and, by the end of observation at 14 days, by discolouration due to blanching of the skin, complete areas of alopecia, and scars. Histopathology should be considered to evaluate questionable lesions.

RESULTS AND DISCUSSION

The physical appearance of various formulations was determined by visual inspectonuner black and white background and all formulation (F1 to F12) were found to be yellowish viscous creamy preparations with a smooth and homogenous appearance. All the selected formulations were found to be homogenous without any aggregates or lumps and were found to be smooth without any grittiness. All the selected formulations were found to be homogenous phase; oil phase and aqueous phase were mixed well without any Phase separation. Spreadability of the formulated gels was determined and all selected gels were found to be easily spreadable and thus shows good patient compliance and good absorption from skin. The

extrudability test was carried out by using Pfizer hardness tester. The test was carried out as described in section. All the selected formulations were found to be easily extrudable from the container. Easier the gel extrudes out, better the patient compliance.

Discussion of Skin irritation test

The selected gel formulations were found to show no redness of skin and no skin irritation. The formulation was found to be safe when topically applied.

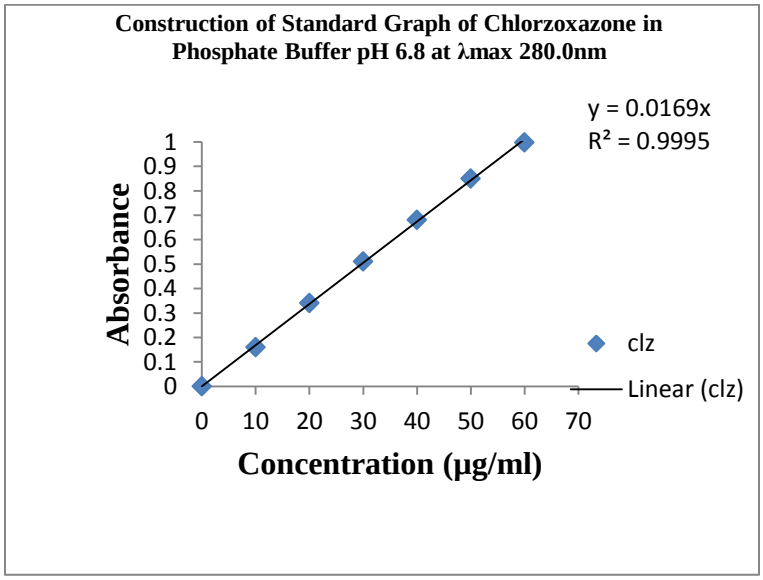
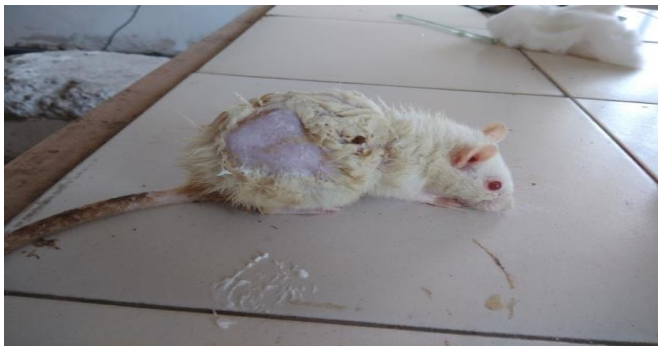
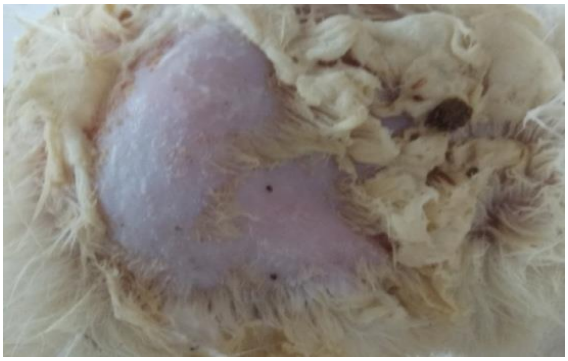


Figure 2: standard graph of Chlorzoxazone in Phosphate Buffer pH 6.8 at λma280.0nm

SKIN IRRITATION TEST





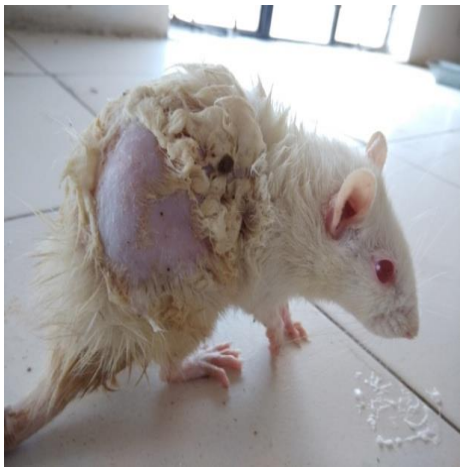


Table1: Results of skin Irritation

S.No	Animal.No	Erythema and Eschar Formation					Oedema Formation					Total
		erythema	Very slight erythema	Well defined erythema	Moderate to severe erythema	Severe erythema	oedema	Very slight oedema	Slight oedema	Moderate oedema	Severe oedema	
1.Control	1	1	-	-	-	-		-	-	-	-	1
	2	1	-	-	-	-		-	-	-	-	1
	3	1	-	-	-	-		-	-	-	-	1
	4	-	-	-	-	-		-	-	-	-	-
	5	1	-	-	-	-		-	-	-	-	1
	6	1	-	-	-	-		-	-	-	-	1
											Average	0833
2.Test	1	1	-	-	-	-		-	-	-	-	1
	2	1	-	-	-	-		-	-	-	-	1
	3	-	-	-	-	-		-	-	-	-	-
	4	-	-	-	-	-		-	-	-	-	-
	5	1	-	-	-	-		-	-	-	-	1
	6	1	-	-	-	-		-	-	-	-	1
											Average	0.667

CONCLUSION

Chlorzoxazone emulgel was prepared by emulgel method by using various excipients such as polymers as Carbopol-940 $ HPMC K4 and cetostearyl alcohol, Arachis oil, Propylene Glycol, Menthol, Glycerol, Tween80, Span20 and with penetration enhancers such as Lemon Grass Oil. The prepared emulgel was evaluated for its properties Further the analysis of release mechanism was carried out by fitting the drug release data to various kinetic equations like Zero order, First order, Higuchi's and korssmeyer peppas equations and from the values so obtained, the best fit model were arrived at. From the above results Formulation F4 was found to be best formulation for the topical release of Chlorzoxazone that complied with all the

parameters. It releases 98.82 % of drug in 12 h time. It show greater faster released in *Ex-vivo* release studies, within one hour 60-70% drug was released. It produces greater flux of drug than marketed formulation.

REFERENCES

[1]. Sunil kumar yadav, Manju kumar mishra, Anupama Tiwari 'A New Approach For Enhanced Topical Drug Delivery', International Journal of Current Pharmaceutical Research .9(I), 2017, 15.

[2]. Bhatt Preeti and Gnanaranjan.G.A.'Novel Formulation Approach For Topical Delivery Of Hydrophobic Drugs', International Journal Of Pharmacy,IRJP .,4(2), 2013 , 12.

[3]. Shiwang Sharma and Arvind Sharma 'Development, Formulation, Characterization And Evaluation Of Elastic Liposomal Formulation Of Chlorzoxazone For Transdermal Delivary', International Journal Of Therapeutic Applications. 2, 2012, 11-12.

[4]. Ansiya M.A, Krishnakumar K, Anish John, Dineshkumar B 'Emulgel With Essential Oil', Health Sciences:An International Journal., 5(1), 2012, 1-5.

[5]. Surender Kumar, Neeraj Singh and Satish Chander Arora 'Emulgel:An Insight' European Journal Of Pharmaceutical And Medical Research, 2015.1168-1171.

[6]. Sunil kumar yadav, Manju kumar mishra, Anupama Tiwari 'A New Approach For Enhanced Topical Drug Delivery', International Journal of Current Pharmaceutical Research.,.9(I), 2017,16.

[7]. Bhatt Preeti, and Gnanaranjan.G.A'Novel Formulation Approach For Topical Delivery Of Hydrophobic Drugs', International Journal Of Pharmacy,IRJP., 4(2), 2013, 12-13.

[8]. Vikas singla, Seema Saini, Baibhav Joshi and A.C Rana.Emulgel 'A New Platform For Topical Drug Delivery', International Journal Of Pharma and Bio Sciences, 3(1), 2012, 488-489.

[9]. Surender Kumar, Neeraj Singh and Satish Chander Arora 'Emulgel:An Insight'. ISSN European Journal Of Pharmaceutical And Medical Research. 2015, 1170-1171.

[10]. Sunil kumar yadav, Manju kumar mishra and Anupama Tiwari 'A New Approach For Enhanced Topical Drug Delivery',International Journal of Current Pharmaceutical Research .9(I), 2017, 16-17.

[11]. Kiran verma,'Formulation and Evaluation Of Aceclofenac Emulgel',Article in Journal Of Advanced Pharmaceutical Technology & Research.3(1), 2015, 0052-0056.

[12]. Manju kumar mishra and Anupama Tiwari 'A New Approach For Enhanced Topical Drug Delivery', International Journal of Current Pharmaceutical Research, .9(I), 2017, 16.

[13]. Pottala Swathi and Kiran Vema (2015) 'Formulation and Evaluation of Aceclofenac Topical Emulgel' International Journal Of Advance Pharmaceutical Science & Innovative Research Development Neeraj Singh and Satish Chander Arora 'Emulgel:An Insight' European Journal Of Pharmaceutical And Medical Research., 3(1), 2013, 173.

[14]. Baibhav Joshi, Seema Saini, and A.C Rana.Emulgel 'A New Platform For Topical Drug Delivery' International Journal Of Pharma and Bio Sciences. 3(1), 2012, 489.