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Formulation and evaluation of a topical emulsion gel of a non steroidal anti-inflammatory drug

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ABSTRACT

The present study was undertaken to formulate and evaluate a topical emulsion-gel of a non steroidal anti-inflammatory drug, choosing Nimesulide as a model drug. Eight formulations were prepared with different concentrations of carbopol 934p as polymer, varying the concentration of tween 80 and adjusting the pH to 6.5 and 8.0.The concentrations of nimesulide and isopropyl myristate were 1% w/w and 10% w/w, respectively in all the formulations. The prepared formulations were evaluated for various physicochemical parameters. The release studies were carried out by dialysis membrane grade 150 and hairless rabbit skin later compared with marketed product. The formulations were evaluated for anti-inflammatory activity in carrageen an-induced rat paw oedema model. Subjective analysis of formulations in healthy human volunteers for acceptability was carried out. FT-IR spectra confirm that there is no incompatibility between drug and excipients. The formulation with 1% w/w carbopol 934 P, 10% w/w tween 80 and pH 8.0 was found to possess maximum percentage drug diffusion comparable to marketed preparation. Maximum anti-inflammatory activity was market preparation. The formulations were stable for 60 days as no significant change in physicochemical characteristics and drug release properties were observed. The formulation was found to be acceptable among healthy human volunteers and is comparable to marketed formulation. From the present work, it can be concluded that Nimesulide can be formulated into topical emulsion-gel with better drug release properties and improved pharmacological effect.

Introduction

Transdermal and topical formulations are becoming increasingly important and their use in therapy is becoming widespread. Although topical products to treat dermatological ailments have existed from earliest times, transdermal products, for which skin is used as an alternate route for systemic therapy, are relatively new[1]. The transdermal permeation of a chemical involves partitioning into and transport through the cutaneous layers, namely the stratum cornea, the viable epidermis (stratum basale) and the upper dermis. But the skin acts as a barrier to topically administered drugs[2, 3]. The mechanism of permeation through the skin is by two possible pathways. The trans-cellular route in which penetrates diffuses directly through cells and the intercellular route, in penetrate diffuses around cells in a tortuous manner. The experimental evidence suggests that the intercellular route pre-dominates the trans-cellular route.

It is known that vehicle effects may have profound influence upon the per-cutaneous delivery from topical products[4]. Co-solvents may alter the barrier properties of the skin[5]. Some substances having considerable polarities also enhance the permeability of the horny layer[6]. Hence besides hydrogels, gels containing both oil and water have been developed. These gels, commonly termed as emulsion-gels, attempt to reduce the barrier properties of the skin, as well as improve the drug release from the topical formulation. Nimesulide belongs to the sulphonanilide group of compounds which rapidly and extensively absorbed after oral administration[7]. Carbomer 934 P is a high molecular weight polymer of acrylic acid cross linked with allyl ethers of sucrose or penta erythritol Carbomers are mainly used in liquid or semi solid pharmaceutical formulations as suspending or viscosity increasing agents[8]. Isopropyl myristate is used as a component of semi solid bases and as a solvent for many substances applied topically[9].

Materials and methods:

Materials

Nimesulide (Eros pharma Ltd, Bangalore), Carbomer (B.P.R.L Ltd, Bangalore.), Dialysis membrane, 150(Himedia Labs, Mumbai), Isopropyl Myristate (Rolex Laboratories Ltd), Triethanol amine (S.R.L. Ltd, Mumbai), Tween 80(Loba chemicals Ltd, Mumbai). All other chemicals were of SR grade.

Methods

Preparation of emulsion –gel

The emulsion – gels were prepared in two steps.

1. Preparation of Carbopol gel

Weighed quantity of carbopol was added to calculated amount of distilled water and stirred well and allowed to soak for 2 hours. Required quantity of triethanolamine was added to form the gel. The required amounts of methyl paraben and propyl paraben were dissolved in ethanol and added to the gel.

2. Preparation of emulsion -gel

Iso propyl myristate and tween 80 were mixed together and nimesulide was added to the mixture. The whole mixture was heated on a water bath to 90^0 C for 25 minutes which ensured complete solubility of nimesulide. An equal portion of the above mixture was added to previously prepared carbopol gels with

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homogenization (3000rpm) for a period of 20 minutes. The homogenization was continued for 45 minutes. The required pH of 6.5 and 8.0 was adjusted by addition of triethanolamine. The measurements of pH were done using systronics digital pH meter. Concentrations of each of the ingredients used in the formulation are reported in Table 1.

The concentrations of Nimesulide (1% $^{w}/_{w}$), isopropyl myristate (10% $^{w}/_{w}$), methyl paraben (0.1% $^{w}/_{w}$), propyl paraben (0.25% $^{w}/_{w}$) and ethanol (5% $^{w}/_{w}$) were identical in all the formulations.

Physicochemical characterization of prepared formulations [11,12,13,14]

Determination of Drug Content

One gram of formulation was placed in 100ml volumetric flask. About 20ml of pH 7.4 phosphate buffer solution was added to the volumetric flask and shaken well. Further, the volume was made up to the mark with pH 7.4 phosphate buffer solution. Suitable dilutions were made and drug concentration was determined by measuring the absorbance at 393nm.

Determination of Viscosity

Method proposed by Lardy F., et al., was followed33. The viscosities of the formulations were determined at 30^0 C ± 1^0 C using Brookfield digital viscometer with spindle T-F at 10 rpm.

Determination of Spreadability

Method proposed by Lardy F., et al., was followed33. The spreadability of emulsion- gels was evaluated at 30^0 C ± 1^0 C with the following conditions: the spreading diameter (ϕ) of 1 ± 0.01 g of emulsion-gel placed between two horizontal glass plates (16 ×16 cm), was measured after 1 minute (mass of upper plate 125 ± 1 g). Following classification was adopted: fluid gel ϕ > 70 mm semi-fluid gel 70 mm ≥ ϕ >55 mm, semi stiff gel 55 mm ≥ ϕ > 47 mm, stiff gel 47mm ≥ ϕ >40 mm and very stiff gel ϕ ≤ 40 mm.

Determination of swelling

One gram of formulation was placed in 100 ml stopper measuring cylinder containing 100 ml of pH 7.4 phosphate buffer solution. The cylinders were left undisturbed for 6 hours. After 6 hours, the volume of pH 7.4 phosphate buffer was measured. The amount of loss in volume of buffer solution was considered as amount taken up by emulsion-gels. Since the weight per ml ratio of water is 1, the amount taken up by emulsion –gels was considered as gain in weight34.

In vitro drug release and permeation studies

Diffusion through dialysis membrane

Emulsion-gel equivalent to 10 mg of nimesulide was placed on dialysis membrane, which was mounted on Franz diffusion cell, the top of which was clamped securely. The receptor medium was pH 7.4 phosphate buffer maintained at constant temperature of 37^0 C± 1^0 C by circulating water bath. The samples were withdrawn from the receptor compartment at predetermined time intervals and replaced with an equal volume of fresh buffer solution. The samples were analyzed for nimesulide content. The market sample of nimesulide gel (Nimulid transgel) was used for comparison.

Diffusion through rabbit skin

Previously cleaned hairless rabbit skin, obtained from slaughter house was allowed to hydrate in pH 7.4 phosphate buffer solution for 1 hour before being mounted on Franz diffusion cell with stratum cornea facing the donor comportment35. On the basis of in vitro release studies conducted through dialysis membrane, samples with optimum drug release were selected. Similar procedure was followed as mentioned for diffusion study through dialysis membrane. The market sample of nimesulide gel (Nimulid transgel) was used for comparison.

Determination of anti- inflammatory activity in rats

Clearance was obtained from Institutional Animal Ethics Committee institutional by J.S.S college of pharmacy, J.S.S mahavidyapeetha mysore-570015, Karnataka, India. before commencement of the study. The anti - inflammatory activity of optimized formulations were determined in carrageenan induced rat paw edema. Three formulations were selected for the study. The market sample of nimesulide gel (Nimulid transgel) served as a positive control.

Animals were segregated into groups each of 4. In treated groups, the formulation was applied topically 30 minutes before injection of carrageenan. In negative control group, no formulations were applied. About 0.05ml of 1% carrageenan suspension was injected in sub plantar region of the paw of all rats. Initial paw volume was measured by using plethysmograph before the commencement of the experiment. The paw volume was measured at of 0.5 hr, 1hr, 2 hr, 3 hr, 4 hr and 6 hr.

Compatibility studies

FT-IR spectroscopy was employed to ascertain the compatibility of nimesulide with the excipients. The individual drug and drug with excipients were separately scanned (with emulsion-gel without drug as blank). Both the spectra were compared for confirmation of common peaks.

Accelerated stability studies

Accelerated stability studies were carried out for optimized three formulations. The formulations were packed in containers, capped securely and kept at 40^0 C for 60days. The drug content of the formulations was analyzed on 15th, 30th 45th and 60th day. The physicochemical parameters like pH, viscosity, spreadability and swelling were analyzed on 0th and 60th day. The drug release and permeation studies were carried out at the end of 60th day.

Subjective analysis in healthy human volunteers

Approval was obtained from the institutional ethical committee by J.S.S college of pharmacy, J.S.S maha-vidyapeetha mysore-570015, Karnataka, India. Before commencement of the study. The optimized formulation was tested for acceptability in healthy human volunteers. Twenty volunteers were selected for the study. Volunteers' consent was obtained before the study. The selection was random and there were no inclusion / exclusion criteria. Market sample (Nimulid transgel) was used for comparison. The study was double blind with cross over design. A questionnaire was prepared to assess the color, cooling effect, spreadability, odor, staining problems and side effects. The volunteers were informed to apply the formulation on unexposed part of their skin and fill the questionnaire supplied. The volunteers were also told to rate the formulation on a 0 – 10 scale.

Result and discussion:

The results were reported in Table 2. It was observed that nimesulide was uniformly distributed in all the formulations with small deviation in formulations F7.

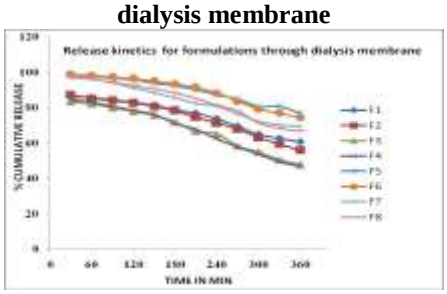
The results were reported in Table 2. The result signifies with increase in the concentration of carbopol, the viscosity was increased considerably. But changes in pH or surfactant concentrations did not appreciably alter the viscosity. The changes were not significant.

The results were reported in Table 2. It was observed that most of the prepared emulsion-gels with carbopol as the polymer

were of semi stiff gel category. From Table 2 it can further be inferred that with increase in polymer concentration the spreadability of the formulations decreased. But change in concentration of surfactant and change in pH did not show any significant effect on spreadability.

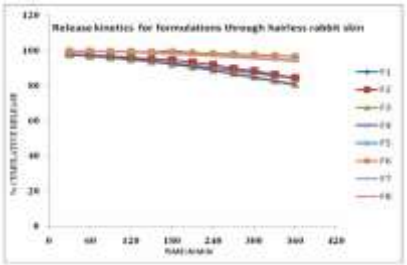
The results were reported in Table 2. From the data it can be observed that the swelling was dependent on the concentration of the polymer.

Figure 1: Release kinetics for formulations through dialysis membrane



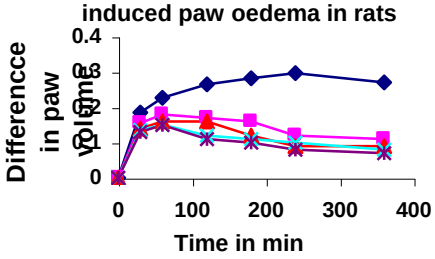
Percentage drug diffused for 6 hr at 30 min interval is reported in Table 2 and Figure 1 Amongst the eight formulations prepared, formulation F4, containing 1%$^{w}/_{w}$ carbopol, 10% $^{w}/_{w}$ tween 80 with pH 8.0 showed the highest diffusion. Formulations F3, F2, F1, F8, F7, F6 and F5 showed diffusion in decreasing order. The concentration of the polymer significantly affected the percentage of drug diffused, with increase in polymer concentration, the drug diffusion was decreased. This may be due to the fact that, the aqueous medium is more and more gellified and is less available for diffusion of nimesulide. This also indicates that, major fraction of nimesulide is in the oil phase. An increase in the concentration of surfactant also increased the drug diffusion, as observed in formulation keeping the polymer concentration constant. Surfactant has increased the solubility of nimesulide, thus a better diffusion was observed. The pH of the formulations had a negligible effect on the diffusion of nimesulide. Nimesulide with pKa value 6.5, was expected to exhibit better drug release/diffusion with increase in pH. Though a small increase in drug diffusion is observed, it was not significant. Higuchi plots revealed that drug release was by diffusion. The plots of release kinetics (log percentage diffused against time) demonstrated that diffusion followed first order kinetics.

Figure 2: Release kinetics for formulations through hairless rabbit skin



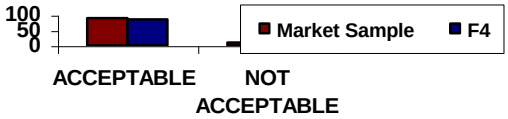
Release kinetics data and profile is reported in Table 3 and Figure 2. Formulation F4, containing 1%$^{w}/_{w}$ carbopol, 10% $^{w}/_{w}$ tween 80 with pH 8.0 showed the highest diffusion and was comparable to that of marketed sample. Formulations F3, F2, F1, F8, F7, F6 and F5 showed diffusion in decreasing order. The pH of the formulations had a negligible effect on the diffusion of nimesulide. It was expected that, at higher nimesulide with pKa value 6.5, can be expected that drug release/diffusion would be increased with increase in pH. Though a small increase in drug diffusion is observed, it was not significant. Higuchi plots revealed that drug release was by diffusion. The plots of release kinetics (log % cumulative retained against time) demonstrated that diffusion followed first order kinetics.

Figure 3 .Anti-inflammatory activity of formulations in caraageenan induced paw oedema in rats



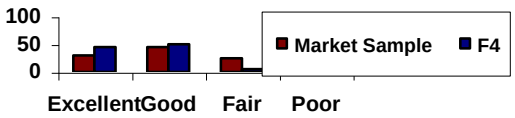
The anti - inflammatory activity was carried out in carrageenan-induced rat paw edema model. Three formulations were selected for the study. The market sample of nimesulide gel was a positive control. The data is reported in Table 4 and Figure 3.

Figure 4 .Percentage response for formulations for acceptability



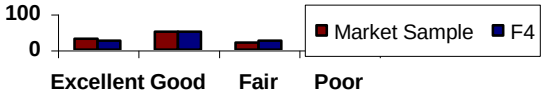
Formulation F4 exhibited highest anti-inflammatory activity followed by F3 and F2. Formulation F4 showed anti-inflammatory activity similar to marketed sample. The results of *in vitro* diffusion studies support the anti-inflammatory activity of the formulations. Nimesulide was compatible with the excipients used, as no significant variation in height, intensity and position of peaks was noticed in both the spectra. It was observed that all the parameters did not show significant changes from initial values and values during and after stability studies.

Figure 5. Percentage response for formulations for cooling effect



Subjective analysis is carried out after obtaining approval of Institution ethical committee. The volunteers were informed to apply the formulation on their unexposed skin and fill the questionnaire. The volunteers were also asked to rate the formulation on a 0 – 10 scale. A comparitive profile of the study is reported in Figure 4-10.

Figure 6. Percentage response for formulations for spreadability



The percentage response indicated that the cooling effect was better for prepared formulation compared to market preparation. The other parameters like spreadability and staining were similar for both the formulations. But the odour of the prepared formulation was not acceptable. Most of the volunteers expressed that odour of formulation to be improved. Side effects were not observed in both the formulations.

Figure 7. Percentage response for formulations for odour

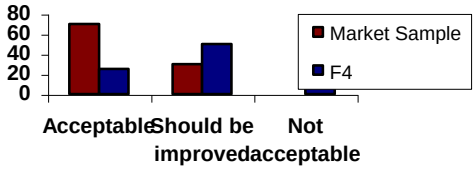


Figure 8. Percentage response for formulations for staining problems

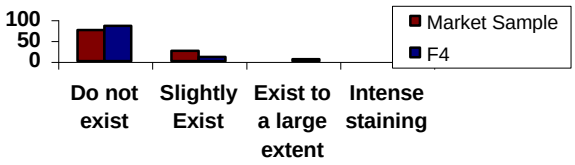


Figure 9. Percentage reponse for formulations for side effects

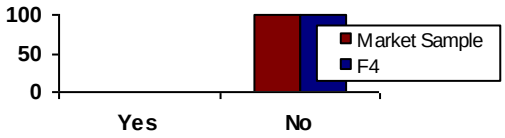
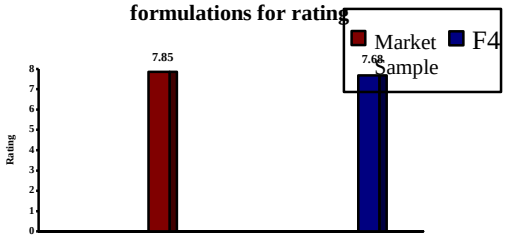


Figure 10. Percentage Response for the formulations for rating



Conclusion:

From the study, it was concluded that Nimesulide can be formulated into topical emulsion-gel with better drug release properties and improved pharmacological effect using % $^{w}/_{w}$ carbopol 934 p, 10% $^{w}/_{w}$ tween 80 and pH 8.0.

Acknowledgement:

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Table 1 Emulsion-gel formulations with varying excipient concentrations

Formulation	Carbopol 934p % $^{w}/_{w}$	Tween 80 % $^{w}/_{w}$	Triethanol amine % $^{w}/_{w}$	Water	pH
F1	1%	8%	1%	Q.S.	6.5
F2	1%	8%	1.25%	Q.S.	8.0
F3	1%	10%	1%	Q.S.	6.5
F4	1%	10%	1.25%	Q.S.	8.0
F5	3%	8%	1%	Q.S.	6.5
F6	3%	8%	1.25%	Q.S.	8.0
F7	3%	10%	1%	Q.S.	6.5
F8	3%	10%	1.25%	Q.S.	8.0

Table 2 Results of drug content, viscosity, spreadability, swelling, %drug diffused and % cumulative release through dialysis dialysis membrane

Formulation No.	Drug content (mg/100ml) Mean ± S.D*	Viscosity in m.Pa.s × 102 Mean ± S.D*	Spreadability (in cm) ± S.D*	Swelling (in Gm) ± S.D*	% drug diffused in 360 min (Dialysis) Mean ± S.D*	% cumulative release through dialysis membrane (360 min)
F1	9.63 ± 0.23	320 ± 0.00	5.4 ± 0.163	0.23 ± 0.047	39.35 ± 0.532	60.65
F2	9.86 ± 0.0791	330 ± 24.4	5.3 ± 0.00	0.26 ± 0.169	44.14 ± 0.387	55.86
F3	9.56 ± 0.0945	290 ± 37.41	5.3 ± 0.141	0.36 ± 0.169	52.10 ± 0.648	47.90
F4	10.03 ± 0.205	316 ± 9.42	5.23 ± 0.186	0.40 ± 0.163	53.23 ± 0.870	46.77
F5	9.73 ± 0.094	1993 ± 87.3	4.8 ± 0.00	1.5 ± 0.294	23.18 ± 0.839	76.82
F6	9.8 ± 0.00	1730 ± 28.28	4.7 ± 0.141	1.63 ± 0.308	25.66 ± 0.255	74.34
F7	9.53 ± 0.20	2000 ± 50.99	4.83 ± 0.134	1.33 ± 0.205	30.58 ± 2.65	69.42
F8	9.86 ±0.188	1943 ± 28.6	4.70 ± 0.213	1.66 ± 0.402	33.32 ± 0.261	66.98
Marketed product	-	-	-	-	55.98 ± 0.727	-

*Standard deviation mean n = 3

Table 3 % drug diffused and % cumulative release through hairless rabbit skin

Formulation No.	% drug diffused in 360 min (Hairless rabbit skin) Mean ± S.D*	% cumulative release through Hairless rabbit skin (360 min)
F1	15.78 ± 0.372	84.22
F2	15,64 ± 0.355	84.36
F3	19.07 ± 0229	80.93
F4	19.61 ±0.531	80.39
F5	3.38 ±0.128	96.62
F6	3.46 ±0.151	96.54
F7	5.48 ±0.237	94.52
F8	6.25 ±0.649	93.75
Marketed product	20.95 ± 0.577	-

Table 4 Data showing anti-inflammatory activity of formulations in carrageenan-induced paw oedema

Formulation No.	Difference in paw volume (ml) at Time(min)					
	30	60	120	180	240	360
Control	0.185	0.227	0.265	0.282	0.297	0.270
F2	0.155	0.180	0.170	0.160	0.120	0.110
F3	0.140	0.160	0.160	0.120	0.090	0.090
F4	0.135	0.151	0.120	0.110	0.100	0.080
Market sample	0.130	0.150	0.110	0.100	0.080	0.070