RESEARCH ARTICLE

Formulation and *in-vitro* evaluation of chrysophanol topical gel

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This study describes the formulation and characterization of topical gel of chrysophanol containing carbopol as gel base. The partition of drug between skin and the hydrogel matrix was considered to play an important role in the permeation process. The effect of three levels of carbopol and three different permeation enhancers on chrysophanol permeability was determined *in vitro*. Each formulation was characterized in terms of viscosity, pH, extrudability, spreadability, homogenicity, drug content, skin irritation, stability, and drug release studies. The gel consisting of 1% carbopol as gel base and 15% dimethylsulfoxide showed superior physicochemical and permeability properties and it was ranked best. Reports of pharmacokinetic parameters showed that the drug release is controlled by both diffusion and relaxation processes.

Key words: *Chrysophanol, diffusion, gel*

INTRODUCTION

Infection is a major problem in wound management. Antibiotic resistance by the pathogenic microorganism renders drug ineffective and calls for improved designing and development of new drugs. New approach has been developed to isolate active components from botanicals and formulate into suitable forms. Chrysophanol (1,8-dihydroxy-3-methylanthraquinone) are naturally occurring compounds that have been isolated from medicinal plants, belonging to various botanical families such as Rhamnaceae (buckthorn, cascara), liliaceae (aloe), Polygonaceae (rhubarbs), and Caesalpiniaceae (senna). In our previous study, chrysophanol isolated from cassia fistula possess potent antimicrobial activity against skin infecting pathogenic organisms.[1] In order to use the potential of chrysophanol, we designed a topical gel formulation of chrysophanol for skin infections which could be successfully applied topically for the management of skin infection.

Different strategies have been proposed to achieve efficient drug delivery systems, and in the last few years hydrogels and gel in general have been considered good candidates for oral, rectal, ocular, cutaneous, and subcutaneous administration; also it has several advantages such as the ease of administration, none greasy, patient compliance, high residence time on the skin, and better drug release and diffusion.[2-5] Among polymers used for formation of gel base, carbomer resins (carbopol-940) are used. Carbopol is a polyacrylic acid derivative. Viscosity of carbopol gel depends on the pH.[4] The release of the drug from topical preparations depends on the physicochemical properties of the vehicle and the drug used. In order to enhance drug release and skin permeation, methods such as coadministration of suitable chemical enhancer have been studied. The aim of the work is to study the use of appropriate concentration of carbopol and suitable permeation enhancer in the chrysophanol release from gel, which may give a gel of suitable physical properties, high drug release consequently, and good bioavailability.

MATERIALS AND METHODS

Preparation of gels

Chrysophanol gels were prepared using carbopol-940 as gelling agents. Solid dispersion of chrysophanol, as described in plan, was prepared using Novel cogrinding method and PEG 6000 along with HPMC as carriers;

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100 mg of drug was mixed with mixture of 100 mg of PEG 6000 and 50 mg of HPMC (2:1) and blunted well in china dish using spatula, and then this mixture was heated over water bath till PEG 6000 melted while persistently mixing the blend. After PEG 6000 melted, the china dish was taken out of the water bath and allowed to cool to room temperature. Mixture of solvent system (3 ml) comprising methanol and dichloromethane (1:2) was added and mixed well. After controlled evaporation of the solvent system, coground precipitates of chrysophanol were collected and dissolved in specified quantity (15% v/w) of permeation enhancer. This solution of solid dispersed drug in permeation enhancer was added to neutralize carbopol (neutralized by triethanolamine) with continuous mixing using high-speed mechanical stirrer. In addition, glycerin and purified water were added to the gel to make the content have 10 g of weight [Table 1].

Evaluation of chrysophanol gel

Physicochemical evaluation of gels

Direct measurements were made using a digital pH meter (MK-IV SYSTRONICS).

Viscosity determination

Viscosities were determined in a cone and plate viscometer (Digital Rheometer model DV11, Brookfield) of the gels prepared. A spindle (no. 7) was rotated at 10 rpm. Samples of the gels (0.5 g) were left to settle over 30 mins at the assay temperature (37°C) before measurements were taken. Viscosities were noted in cps as mean of triplicate.

Spreadability

Spreadability was determined using a spreadability apparatus.[6] After applying weight, time in seconds required to separate the slides was noted. Spreadability of each formulation was reported in seconds. Spreadability was then calculated by using the formula:

$$S = M.L/T \quad (1)$$

where S=spreadability, M=weight tide to upper slide, L=length of glass slide, and T=time taken to separate the slide completely from each other.

Extrudability

Extrudability, using an extrudability apparatus.[7] A closed collapsible tube containing formulation was pressed firmly at the crimped end. When the cap was removed, formulation extruded until the pressure dissipated. Weight in grams required to extrude a 0.5 cm ribbon of the formulation in 10 seconds was determined. The average extrusion pressure in grams was reported.

Homogeneity test

The formulations were tested for their homogenicity by visual appearance after the gels have been set in the container. Also a small quantity of each gel is pressed between the thumb and the index finger, and the consistency of the gel is noticed whether homogeneous or not.

Drug content analysis

For the estimation of the drug in gels, chrysophanol was extracted from 1 g of each gel formulation with 50 ml of methanol for 30 min, and the resultant mixture was filtered through membrane filter (pore size 0.45 μm). From this, 2.5 ml was pipette out and made up to 10 ml. Then 1–10 ml, the absorbance of the sample was determined spectrophotometrically at 225 nm. The concentration of chrysophanol was estimated from the regression equation of calibration curve.[8]

Primary skin irritancy test

The protocol of the study was approved by the Ethical Committee of Amrita University. Six healthy, previously unused New Zealand White rabbits of either sex and free from skin irritation or other dermatological lesions (weighing 2.0–2.5 kg) from Experimental Animal Centre of Amrita University Cochin, India, were chosen for this study. The animals were individually housed in stainless steel cages. The back of each animal was clipped free of fur with an electric clipper at least 4 h but no more than 24 h before testing. A 50 mg portion of chrysophanol gel was applied to two sites (one site intact and one site abraded) on each rabbit under a double layer of gauze in an area of skin approximately 1 × 1 inch square. After 24-h exposure, the gauze was removed. The test sites were gently sponged with deionized water in an attempt to remove any remaining test article residue. The

Table 1: Composition for the batches of gels

Composition	C11	C12	C13	C14	C21	C22	C23	C24	C31	C32	C33	C34
Chrysophanol (g)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Carbopol (g)	1	1	1	1	1.25	1.25	1.25	1.25	1.5	1.5	1.5	1.5
DMF% v/w	–	15	–	–	–	15	–	–	–	15	–	–
DMSO% v/w	–	–	15	–	–	–	15	–	–	–	15	–
PEG 400% v/w	–	–	–	15	–	–	–	15	–	–	–	15
Glycerine % v/w	10	10	10	10	10	10	10	10	10	10	10	10
Triethanolamine (ml)	2	2	2	2	2.5	2.5	2.5	2.5	3	3	3	3
Methylparaben (w/v) ml	2	2	2	2	2	2	2	2	2	2	2	2
Propylparaben (w/v) ml	1	1	1	1	1	1	1	1	1	1	1	1
Deionized water to produce (g)	10	10	10	10	10	10	10	10	10	10	10	10

test sites were evaluated 24 and 72 h following removal of the gauze. Each test site was examined for dermal reaction in accordance with the Draize scoring criteria. The primary irritation index (the sum of the scored reactions divided by a factor representing the number of scoring intervals multiplied by the number of test parameters multiplied by the number of rabbits) was calculated following test completion.[9,10]

Stability evaluation

Stability studies were performed for 6 months. Samples (packed in glass vials) were prepared in triplicates and were kept at two stability testing conditions, viz. 4–8°C serving as control and 30°C/70% RH (Stability Chamber, Remi-2K) serving as test condition as per ICH Guideline. Stability samples were evaluated for drug content, pH, homogeneity, and viscosity at each sampling point (0, 3, and 6 months).

In vitro drug release studies

The *in vitro* drug release from gel formulations was studied across cellulose membranes (Sigma Aldrich) using Franz-type diffusion cells (Orchid Scientifics-FDC-06) with effective diffusional surface area of 1.54 cm2. The cellulose acetate membrane (cellophane membrane) having a pore size 0.45μ was mounted between the donor and receptor compartment of the diffusion cell.[11-13] The receiver compartment was filled with 15 ml of phosphate buffer pH 7.4 to ensure sink condition. The donor compartment of the cell was filled with 1 g vehicle containing the test drug. The system was maintained at 37±0.5°C by a water bath circulator and a jacket surrounding the cell, with stirring at 600 rpm throughout the experiment. One milliliter sample was withdrawn at intervals of 1 h for a period of 12 h, and each time equal volume was replaced with drug-free receptor fluid.[14,15] All samples were analyzed by UV spectrophotometer at 225 nm. The experiment was carried out in triplicate, and the mean cumulative percentage releases from three batches were calculated.

The following pharmacokinetic parameters are calculated:

Cumulative quantity of chrysophanol collected in the receiver (μg/cm2) was plotted as a function of time. The flux value (Jss, μg/cm2/h) for each experiment was obtained from the slope (steady-state portion) of the linear portion of the data fitted by regression analysis.[16] For release data analysis, cumulative permeation (μg/cm2) was plotted as a function of square root of time, where linearity is indicative of diffusion controlled drug release.[17] Release rate was estimated as the slope of such plots (μg/cm2/t0.5). Penetration-enhancing activities were expressed as enhancement ratios (ER), i.e., the ratio of the flux value with enhancer to that obtained without enhancer. Data were subjected to statistical analysis using two-way analysis of variance with *post hoc* test using Graphpad.

RESULTS AND DISCUSSION

All developed gel showed good homogeneity with absence of lumps and pH of all the formulations was found to be between 6.5 and 6.9, which indicated suitability of the formulations for application on the skin. Rheological properties such as viscosity, spreadability, and extrudability of the developed gel formulations were found to be equivalent to marketed gel [Table 2].

The drug content of the gel formulations was in the range of 98.7–99.6%, showing content uniformity. Primary skin irritation study demonstrated that chrysophanol gel cannot be considered a primary irritant to the skin [Table 3].

During the stability studies, the drug content of all the formulations was found to be in good agreement with the theoretical value. The appearance was homogeneous and there was no significant change in pH and viscosity as a function of time for all formulations, indicating the stability of the drug in the formulations [Table 4]

Carbopol, a polyacrylic acid derivative and a well-known compound for its excellent gel property such as sparkling clarity, spread ability, etc., is a weakly acidic compound, and

Table 2: Physicochemical evaluation of gel (*n*=3)

Formulation code	Drug content±SD	Extrude ability, g±SD	pH±SD	Homogenicity	Viscosity in cp at 10 (RPM)±SD	Spreadability (g.cm/s)±SD
C11	99.1±0.04	254±0.2	6.9±0.00	Homogenous	85610±0.00	6.3±0.5
C12	99.1±0.02	243±0.7	6.9±0.00	Homogenous	85646±0.00	6.1±0.7
C13	98.9±0.02	254±0.3	6.9±0.00	Homogenous	85645±0.00	6.2±0.5
C14	99.5±0.05	251±0.4	7.0±0.00	Homogenous	85640±0.00	6.2±0.6
C21	99.1±0.04	272±0.6	6.9±0.00	Homogenous	86290±0.00	6.4±0.8
C22	99.2±0.03	263±0.5	6.5±0.00	Homogenous	86297±0.00	6.3±0.6
C23	98.7±0.03	262±0.6	6.9±0.00	Homogenous	86296±0.00	6.2±0.6
C24	99.0±0.01	259±0.5	6.9±0.00	Homogenous	86289±0.00	6.2±0.9
C31	99.6±0.06	279±0.7	6.6±0.00	Homogenous	80114±0.00	6.4±0.4
C32	99.5±0.05	269±0.3	6.7±0.00	Homogenous	80140±0.00	6.4±0.2
C33	99.4±0.04	268±0.5	6.6±0.00	Homogenous	80137±0.00	6.3±0.7
C34	99.3±0.03	270±0.5	6.8±0.00	Homogenous	80133±0.00	6.2±0.6
Marketed gel (voveran gel)	97.4±0.02	269±0.6	6.7±0.00	Homogenous	85412±0.00	6.0±0.2

when dispersed in water and neutralized carbomer uncoils its chain and results in the gelling that, in turn, increases the viscosity of the solution. Although carbopol has pH-dependent gelling, its gel characteristic is least likely to be influenced by the chance in the pH after its neutralization except in some cases where the change in the pH is due to the presence of incompatible ions. The peculiar structure of neutralized carbapol gel could be prime cause for the semi-rigid gel consistency of the carbapol gels and controlled drug release when drug molecules are incorporated into the gel matrix.[4] Water and drug diffusion from glassy polymers often deviates from the prediction of Fick's law, leading to anomalous or non-Fickian diffusional behavior. The deviation from Fickian behavior has been associated with the finite rate at which the polymer structure rearranges, to accommodate water molecules, and has been observed for many hydrophilic polymer systems. Depending on the dynamics of polymer swelling and the relative mobility of drug and water, Fickian and non-Fickian drug transport may be observed.[18,19]

Since the drug release from the carbapol-based gel is chiefly controlled by both diffusion and polymer relaxation process,[19] the *in vitro* kinetic data are subjected to log–log transformation plots (Peppas model) after being subjected to Higuchi's classical diffusion model to find diffusivity of the drug substance.[17-19]

When the concentration of carbopol was kept at 1%w/w (C11), the amount of drug release was found to be 257.96 μg/cm2/h, at the same concentration, in presence of permeation enhances the rate of release and diffusivity of the chrysophanol molecules were substantially increased [Figure 1]. As suggested earlier, although the drug release is controlled by both diffusion and relaxation processes, before proceeding with data treatment according to drug release model, the *in vitro* kinetic data are subjected to Higuchi's classical diffusion model and a linear relationship (r=0.987-0.985) was observed between the amount released and square root of time as proposed by the Higuchi's theory,[17] indicating the diffusion controlled mechanism of drug release. After that the data were fitted in log–log transformation plots (Peppas model) whose exponent value suggests the additive factors controlled release pattern. The slope values of the Peppas plot are given in the additive factors controlling release pattern. All the slope value range from 0.934 to 1.04 [Table 5] reveals the fact that the drug release either follows Fickian diffusion in zero-order pattern, however, controlled by relaxation processes in which polymer chains are bound to extend and result in increased inter polymer chain spaces that will reduce diffusional resistance the drug release leading to case-II transport.[18,20]

Permeation enhancers, in general, are reported to act by any one of the following mechanism:

1. They act as cosolvent for the drug molecules and increase the solubility of the drug in the matrix. Increase in the solubility, according to Higuchi, will result in increased thermodynamic activity of the drug, which, in turn, results in higher rate of drug release.
2. They reduce the extent of impediment for the release through the barrier membrane, skin, by altering the structure and increasing the fluidity of the skin lipids and keratinized skin layers.

Table 3: Dermal observations

Rabbit number	Reaction	24 h		74 h	
		Intact	Abraded	Intact	Abraded
1	Erythema	0	0	0	0
	Edema	0	0	0	0
2	Erythema	0	0	0	0
	Edema	0	0	0	0
3	Erythema	0	0	0	0
	Edema	0	0	0	0
4	Erythema	0	0	0	0
	Edema	0	0	0	0
5	Erythema	0	0	0	0
	Edema	0	0	0	0
6	Erythema	0	0	0	0
	Edema	0	0	0	0

Primary irritation index:0/24,0/74=0

Table 4: Stability studies of optimized gel formulation C12 (n=3)

Storage and parameters	Period of studies in months		
	0	3	6
4°C±3°C			
Drug content	99.1±0.02	98.9±0.02	98.1±0.04
pH	6.9±0.00	6.9±0.00	6.9±0.00
Homogeneity	Homogenous	Homogenous	Homogenous
30°C±2°C/60% RH±5%RH			
Drug content	99.1±0.01	98.7±0.02	97.9±0.03
pH	6.9±0.00	6.9±0.00	6.9±0.00
Homogeneity	Homogenous	Homogenous	Homogenous

Table 5: *In vitro* kinetic parameters of carbopol-based gel formulations

Formulation code	Flux (µgcm-2h-1)	Diffusivity (µgcm-2h-1/2)	Peppas slope (n)	Enhancement ratio (ER)
C11	257.96	856.71	0.99	1.00
C12	505.69	1734.22	1.07	1.96
C13	453.56	1568.00	1.00	1.76
C14	418.54	1442.05	1.01	1.62
C21	228.02	745.98	0.951	1.00
C22	463.00	1594.93	1.03	2.03
C23	410.59	1416.48	1.04	1.80
C24	345.79	1196.28	1.0	1.51
C31	187.22	646.64	0.936	1.00
C32	385.33	1318.44	0.934	2.05
C33	318.64	1093.57	0.971	1.70
C34	263.28	910.06	0.963	1.41

However, in *in vitro* conditions particularly when the study is conducted using synthetic membranes, the first mechanism determines the rate and extent of permeation enhancement.

Concluding from many reports on similar studies, the increase in the thermodynamic activity of the drug molecules within the matrix will make the permeation enhancement. The extent of increase in the thermodynamic activity, directly dependent of solubility of drug entities, must therefore be in direct correlation with the extent of polymer relaxation. Hence, if the magnitude of the solubility enhancement by a permeation enhancer is more, then, it is likely that, the magnitude of drug release through relaxation process will also be more. The rate of drug release by diffusion depends on, as suggested by Higuchi, many factors that have prime or additive influence, which are listed below.[17]

1. Diffusivity of the drug molecules.
2. Saturation solubility of the drug substance in the dissolution media.
3. Concentration gradient of the drug substance existing between matrix and dissolution media.
4. Porosity of the polymeric matrix.
5. Tortuosity of the polymeric matrix.

While first three of the four mentioned factors depend on the solubility of the drug, the remaining factors, porosity and tortuosity of the matrix, depend on relaxation of polymer chain and solubility of the matrix forming polymer. Hence, it is reasonable to conclude that diffusion and relaxation are additive mechanisms yielding synergistic increments to the extent of drug use.

Enhancement ratio was calculated using both total amount of drug release and flux by comparing formulations with penetration enhancers to control formulation having same polymer concentration, however, with no penetration enhancers. These values divulge following interpretations:

1. The order of influence of permeation enhancers was coherent and reproducible in different polymer concentrations, also, they may be arranged as DMF>DMSO>PEG-400
2. Both flux and diffusivity of the drug molecules were found to decrease in the forementioned order. Probably, this could be described to the subsequent reduction in the ability of the permeation enhancers to increase the drug solubility, in fact thermodynamic activity [Table 5].

CONCLUSION

In the present work, efforts have been made to study the influence of different permeation enhances such as DMF, DMSO, and PEG-400 in promoting the rate of permeation of chrysophanol an antimicrobial drug, in carbopol. The results can be summarized as

1. Drug release studies done with different permeation enhancers and different concentration of carbopol base reveal that DMF had high degree of enhancement for chrysophanol in 1% carbopol gel.
2. Physiochemical evaluation of tests avowed the fact that no formulation has irritancy, in addition to having excellent physical characteristic that may aid large-scale processing of formulations and handling.

REFERENCES

1. Deepa TV, Kavitha RD, Gopalakrishnan S, Sreekanth SK, Shekar S. The Potential of Aqueous and Isolated Fraction from Leaves of Cassia fistula Linn as Antibacterial Agent. Int J Chem Sci 2009;7:4,2363-2370.
2. Ratner B, Hoffman A. Synthetic hydrogels for biomedical application. Hydrogels Medical, Related, Applications. ACS, Symposium Series, No. 31, In: Andrade JD, ed.Andrade JD.Washington,DC:Am.Chem. Society1976;1-36.
3. Peppas N, Bures P, Leobandung W, Ichikawa H. Hydrogels in pharmaceutical formulation. Eur J Pharm 2000;50:27-46.
4. Kumar S, Himmelsten K. Modification of *in situ* gelling behavior of carbopol solution by Hydroxypropyl methyl cellulose. J Pharm Sci 1995;84:344-8.
5. Jones DS, Irwin CR, Woolsen AD, Djok J, Adams V. Physicochemical Characterization and Preliminary Invivo Efficiency of Bioadhesive Semisolid Formulations Containing Flubiprofen for Treatment of Ginjivitis. J Pharm Sci 1999;88:592-8.
6. Mutimer MN, Riffkin C, Hill JA, Glickman ME, Cyr GN. Modern ointment base technology. II. Comparative evaluation of bases. J Am Pharm Assoc Sci 1956;45:212-8.
7. Liberman HA, Rieger MM, Banker GS. Pharmaceutical Dosage Form, Disperse Systems. Vol. 3. New York: Marcel Dekker; 1989. p. 594.
8. Deepa TV, Kavitha RD, Gopalakrishnan S, Ravikumar KG, Sundaram KR, AneeshTP. Development and Validation of Spectrophotometric Method for Chrysophanol in Gel Formulations. Int J Drug Dev Res 2010;2:2.
9. Marzulli FN, Maibach HI. Advance in Modern Toxicology. Vol.4. London: Hemisphere Publishing Corporation; 1997. p. 193-210.
10. Sperling, F. Toxicology Principles and Practice. Vol. 4. New York: WileyInterscience Publication; 1984. p. 168-77.
11. Suwantong O, Opanasopit P, Ruktanonchai U, Supaphol P. Electrospun cellulose acetate fiber mats containing curcumin and release characteristic of the herbal substance. Polymer 2007;48:7546-57.
12. Taepaiboon P, Rungsardthong U, Supaphol P. Vitamin-loaded electrospuncellulose acetate nanofiber mats as transdermal and dermal therapeutic agents of vitamin A acid and vitamin E. Eur J Pharm Biopharm 2007;67:387-97.
13. Siddaramaiah, Kumar P, Divya K, Mhemavathi B, Manjula D. Chitosan/ HPMC Polymer Blends for Developing Transdermal Drug Delivery Systems. J Macrom Sci Part A- Pure and Applied Chemistry. 2006;43:601-7.
14. Mei Z, Chen H, Wang T, Yang Y, Yang X. Solid lipid nanoparticle and microemulsion for topical delivery of triptolide. Eur J Pharm Biopharm 2003;56:189-96.
15. Shah KA, Date AA, Joshi MD, Patravale VB. Solid lipid nanoparticles (SLN) of tretinoin: potential in topical delivery. Int J Pharm 2007;345:163-71.
16. Komatsu H, Suzuki M. Percutaneous absorption of butylparaben through guinea pig skin *in vitro*. J Pharm Sci 1979;68:596-8.
17. Higuchi WI. Analysis of data on the medicament release from ointments. J Pharm Sci 1962;51:802-4.
18. Peppas NA. Analysis of Fickian and non-Fickian drug release from polymers, Pharm. Acta Helv 1985;60:110-11.
19. Peppas NA, Sahlin JJ. A simple equation for the description of solute release. III. Coupling of diffusion and relaxation Int J Pharm 1989;57:169-72.
20. Hadjiioannou TP, Christian GD, Koupparis MA, Macheras PE. Quantitative Calculations in Pharmaceutical Practice and Research. New York: VCH Publishers Inc; 1993. p. 345-8.

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