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Preparation and Evaluation of Clarithromycin Loaded Anti-Microbial Gel for the Treatment of Acne

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Abstract

Objectives: The aim of current study was to design and evaluate different formulations of topical gel containing clarithromycin (CLR) and lactic acid for the treatment of acne and acne scar. The gel was formulated using different concentrations of cabopol-934, propylene glycol and optimized for variable i.e. starring speeds and temperature conditions etc. Five different formulations were prepared and characterized for pH, colour, drug content, spreadability, homogeneity and viscosity. FTIR and DSC of formulation were carried out to confirm drug-excipients compatibility.

Result: The drug content of the formulation was found to be 97.78±0.55%; In-vitro drug release study was performed using Franz diffusion cell and was found to be 98.19±2.43 % in 60 min. and showed the good anti-acne efficacy.

Conclusion: Formulation showed good spreadability, consistency, homogeneity and antibacterial activity indicating its suitability for topical use for acne treatment.

Keywords: Carbopol-934; Clarithromycin; DSC; FTIR; Lactic acid; Topical gel

Abbreviations: CLR: Clarithromycin; MAC: Mycobacterium Avium Complex; AHA: Alpha Hydroxy Acids; TEA: Triethanolamine; RD: Relative Density; ANOVA: Analysis of Variance; SEM; Scanning Electron Microscopy; DMM: Dialysis Membrane Method; ZOI: Zone of Inhibition.

Introduction

Acne is common disease of piloseabaceous units (hair follicles and associated oil/sebaceous glands) experienced by 70%-80% of people between 12-30 years of age and upto 5% of older adults [1,2]. Various factors are responsible for acne like increased sebum production from sebaceous glands under the influence of androgen (dihydrotestosterone) as a result sensitivity of androgen receptors increases; comedones formation due to alteration in keratinisation process; inflammatory mediators released caused neutrophilic and lymphocytic inflammatory response into the skin, follicular bacterial colonisation with *Propionibacterium acnes* [3,4], which stimulates interleukins-12, interleukins-8 and tumour necrosis factor types of pro-inflammatory mediators [5]. *Propionibacterium acnes* are Gram-positive bacillus, an anaerobic, diphtheroid, aero-tolerant bacteria. It is an essential part of the typical flora of skin of human being. The sebaceous follicles of skin, conjunctiva, intestinal tract, oral cavity and the external auditory canal of ears are its natural habitats [6]. *P. acnes* can survive in anaerobic conditions up to 6-8 months *in-vitro*, having slow and unpredictable growth

in aerobic environment [7]. Seborrhoea (excess grease), non-inflammatory open comedones (blackheads), closed comedones (whiteheads), inflammatory lesions (papules, pustules, cysts and nodules) are the clinical characteristics of acne which affect face, neck, upper chest, shoulders and back due to presence of highest piloseabaceous units in these areas [8,9].

Both oral and topical routes of drug delivery are effective for the management of acne but oral route is preferred in severe condition of acne with adjuvant topical therapy [10]. On the other hand, in the mild or moderate conditions of acne topical therapy is considerable. Topical agents such as benzoyl peroxide, retinoids, salicylic acid, azelaic acid and antibiotics alone or in combination are used for effective management and treatment of acne [11].

CLR (6-O-Methylerythromycin) is a macrolide broad spectrum antibiotic kills or inhibits the growth of microorganisms by binding with 50s subunit of ribosomes and inhibit protein synthesis, it is used to treat soft tissue, skin and respiratory tract infection [12]. It is *in vitro* effective against variety of anaerobic/aerobic, Gram-positive/ Gram-negative microorganisms and microorganisms of the *Mycobacterium avium* complex (MAC) i.e. *Pasteurella multocida, Staphylococcus aureus*, *Streptococcus Pyogenes, Streptococcus pneumoniae*, *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Mycoplasma pneumonia, Moraxella catarrhalis*, *Chlamydia pneumoniae*, *Mycobacterium Intracellulare, Mycobacterium Avium*, *Bordetella pertussis*, *Clostridium perfringens, Legionella pneumophila* and *Propionibacterium acnes* [13].

Lactic acid ($C_3H_6O_3$) is a member of alpha hydroxy acids (AHA) used in various types of skin diseases such as ichthyosis, xerosis, seborrheic keratosis, warts and actinic keratosis and also used in chemical peeling for treatment of mottled pigmentation of photodamaged skin, roughness and wrinkling [14,15]. Due to its antioxidant property lactic acid used in repigmentation of vitiligo [16], and act as an antibacterial agent in topical formulations [17]. On the topical application, it enhances the secretion of vascular endothelial growth factor by human reconstructed epidermis which is multifunctional angiogenic cytokine involved in angiogenesis and wound healing [18]. In present research a topical gel formulation was prepared which contain CLR as antibacterial agent and lactic acid as peeling agent for the treatment of acne.

Materials and Methods

Materials

CLR was received as a gift sample from Aurobindo Pharma Ltd, Hyderabad. Carbopol 934, Sodium alginate and Lactic acid were purchased from S.D. fine chemicals Pvt India. Triethanolaminen, Methyl paraben and Propyl paraben were purchased from Sigma Aldrich, Pune, India. *P. acne* (MTCC 1951) was purchased from MTCC Chandigarh, India. Ethanol, glutaraldehyde and other chemicals were used of analytical grade.

Method of Preparation of Gel

CLR-gel was prepared by dispersion method which involves the rapid diffusion of drug across the polymer. 2% w/v carbopol 934 (used as gelling agent) was dispersed in a calculated amount of distilled water with continuous stirring at 800 rpm for 12 h to complete hydration. CLR was dissolved in methanol and added to the dispersion. Then 10% Lactic acid (anti-scar agent), 0.03% Methyl paraben and 0.03% Propyl paraben (preservatives), 3% Propylene glycol (humectants) were added to above preparation and neutralized by triethanolamine (TEA) [19]. Composition of different CLR-gels is presented in Table 1.

S. No.	Ingredients	F_1	F_2	F_3	F_4	F_5
1	CLR	1	1	1	1	1
2	Propylene Glycol	2	3	4	3	3
3	Carbopol 934	1	1	1	2	3
4	Lactic acid	10	10	10	10	10
5	Methyl paraben	0	0	0	0	0
6	Propyl paraben	0	0	0	0	0
7	Triethanolamine	0.5	0.5	0.5	0.5	0.5
8	Distilled Water	q.s.	q.s.	q.s.	q.s.	q.s.

Table 1: Formulation of CLR gels using different gelling agents (quantity in %).
q.s. = Quantity sufficient

Physiochemical Characterization of CLR Loaded Gel

Transparency, Homogeneity, Smoothness and Relative Density: Transparency and homogeneity of the gel was evaluated by visual inspection. 5 ml of gel was taken in test tube and observed for appearance and presence of any aggregates or lumps. Smoothness of gel was observed by rubbing the gel between figures and relative density was determined using relative density (RD) bottle, by comparing the weight in grams of gel formulation (1 ml) with equivalent weights of distilled water [19].

Rheology: The viscosity of CLR-gel formulations was determined at 25±1°C using Brookfield digital viscometer (Model No. DV-E). The gel was rotated at 20 and 30 rpm with spindle no. S-64. At each speed, the corresponding dial readings were noted and corresponding viscosity was calculated [19].

pH: 1 g of CLR-gel was dissolved in 100 mL freshly

prepared distilled water and stored for two hours. pH of aqueous dispersion was determined using digital pH meter. Standardized with standard buffer pH 4.0 & 7.0 [20].

Spreadability: Parallel plate method was used for spreadability test. Briefly 500 mg formulated gel was placed in a circle of 1 cm diameter on glass plate, covered by another glass plate for 5 min with 500 gm weight. Change in the diameter of gel due to spreading of formulation was noted down [21]. Spreadability (S) was calculated using equation (i):

$$S = \frac{M.L}{t}$$ Equation (i)

Where; M = weight (g) tied to the upper glass slide, L = length (cm) moved on the glass slide, t = time (sec)

Drug Content: 500 mg of CLR-gel was weighed and dissolved in 100 ml methanol in conical flask and stirrer for 2 h on mechanical stirrer to completely dissolve CLR. The resulting solution was filtered through Whatman filter paper and analyzed at 226 nm using an UV spectrophotometer [22].

In-vitro Drug Release Study: Weight CLR-gel containing 100 mg CLR and plain CLR drug were placed in a dialysis bag (dialysis membrane-50, HiMedia, Mumbai). Both the ends of dialysis membrane were subsequently sealed and introduced into 15 ml of receptor medium (PBS buffer pH 5.5) separately. Maintained 25±1°C temperature and stirring speed 200 rpm by using magnetic stirrer throughout the study, then 2 ml samples were withdrawn from receptor medium at predetermined time interval and analysed for CLR in plain drug compartment and formulation containing compartment using UV spectroscopy method at 226 nm. All experiments were conducted in triplicate [23].

Characterization of Optimized Gel

Stability Study: The optimized CLR-gel formulation was subjected to accelerated stability studies. CLR-gel was placed in glass vials and subjected to different environmental conditions i.e. 4±2°C and 25±2°C/60±5% RH for 3 months. The samples were evaluated for appearance, clarity, pH and residual drug content after periodic interval of one month [24].

Ex-vivo Studies

Anti-bacterial Study: The antibacterial activity of drugs and drug formulations were determined by agar well diffusion method using *P. acne* as test organism. 100 µl 1% drug solution and prepared CLR-gel were introduced in the well of each plate. The plates of *P. acne* were incubated anaerobically at 37°C for 24 h. The antibacterial activity was evaluated by measuring the diameter of zones of inhibition with the help of a template. The experiment was done in triplicate and the mean was calculated by One-way analysis of variance (ANOVA) [25].

Scanning Electron Microscopy (SEM): SEM images were taken to evaluate the effect of antibiotic CLR on bacteria (*P. acne*) growth. *P. acne* at a concentration of 2µl was suspended in the media. Sample S_1 (untreated) and S_2 (treated with CLR-gel) was incubated for 24 h. After incubation the samples was centrifuged for 3 min at 8000 rpm and then samples were treated with 0.25% gluteraldehyde at room temperature. The samples of *P. acne* were dehydrated with graded ethanol series 20%, 50%, 70%, 80%, 90% for 10 min and one hour with 100% ethanol. After this process the samples were incubated at room temperature for 24 h. Samples for the scanning electron microscopy were coated with silver coating, analysed by NOVA NANOSEM 450 and imaged with software xT microscope Control v6.3.0 build 3066 supervisor [26].

Results and Discussion

Topical drug delivery systems for the treatment of skin disease offer several advantages over oral drug delivery systems. In case of CLR it is well reported that it is associated with various side effects such as stomach pain, vomiting, mild diarrhoea and unpleasant taste etc. when given by oral route and very less concentration of drug reaches to the target site. We hypothesised to design topical drug delivery system for the targeted delivery of drug CLR for acne treatment.

CLR-gel was formulated by employing carbopol 934 as gelling agent and delivery vehicle, these gel systems enhancing the drug skin residence time, drug penetration into skin and skin moistening effect as well as therapeutic efficacy of drug.

Physiochemical Characterization of Optimized CLR-Gel

Among the all five formulations as presented in Table 1, F_1, F_2 and F_4 were found to be transparent, clear and colourless when observed in light, while formulation F_3 and F_5 were white to off white in colour and lumpy in texture. This character may be due to higher concentration of polymers i.e., propylene glycol and carbopol. Similar results have been reported by Tan et al. that the increase the concentration of carbopol in polyethene glycol gel, increased the a general loss of consistency with increased shearing stress [27]. On the basis of these physiological characters F_1, F_2 and F_4 were selected for further studies.

After that, formulations F_1, F_2 and F_4 were compared on the basis of pH and % drug content. The pH of topical formulations is one of the important character and formulation should possess almost same pH as skin. The pH values of all selected formulations in the range of 6.14 to 7.18, which is very similar to pH of outer surface of human skin and i.e. pH 5.4-6.8. On the basis of pH, formulation F_2 and F_4 were found to

be more appropriate for topical preparation which cause no or negligible skin irritations. Zheng et al. previously found that the pH (6.90.2) of gel formulations was compatible with skin and produced no irritation [28].

Viscosity is an important factor in evaluating the overall efficiency of transdermal/topical formulations. The viscosity of the formulations was found to be increased upon increasing the concentration of polymer (carbopol 934 from 1-3% and propylene glycol from 2-4%). Khan, et al. reported that increase in concentration of carbopol increased the viscosity of gel formulation [29]. It might be because polymer makes the compact network in the aqueous phase, which ultimately leads to the formation of gel. The viscosity between 3800-3900 cps is reported to be an ideal viscosity value for topical gel formulation developed using carbopol polymers [30]. Further, spreadability also one of the Important behaviour of topical drug delivery systems like gel or cream, it provide the facts about the force required for gel comes out from the tube.

Suitable viscosity (3853.67±1.23 cps) and spreadability (4.5±0.02) gcm/sec were observed for formulation F_4 at 2% w/v concentration of carbopol 934 and 3 % concentration of propylene glycol with maximum drug content (97.95±0.55%). 2% w/w carbopol and 3% w/w propylene glycol concentration selected as optimized concentration because above this concentration gel converted into lumps or tacky film and required much more force to come out from the tube. Results of characterization of CLR-gel are presented in Table 2. On the basis of the above parameters formulation F_4 was selected for further studies.

S. No.	Formulation	Colour	Texture	pH	Spreadability (gcm/sec)	Viscosity (cps)	Drug content (%)
1	F_1	Transparent	Clear colourless gel	7.18±0.02	5.1±0.04	3124.33±2.51	95.94±0.04
2	F_2	Transparent	Clear colourless gel	6.43±0.16	4.7±0.31	3529.18±1.47	96.55±0.51
3	F_3	White	Lumpy gel	6.51±0.21	3.4±0.24	4279.25±2.51	97.11±0.53
4	F_4	Transparent	Clear colourless gel	6.62±0.13	4.5±0.02	3853.67±1.23	97.95±0.55
5	F_5	White	Lumpy gel	6.14±0.032	2.8±0.06	6108.33±0.95	95.34±0.43

Table 2: Characterization results of CLR-gels.

Drug-Excipients Compatibility

Drug-excipients compatibility was determined by FT-IR analysis. FT-IR analysis revealed that there was no major change obtained in the position of peaks in the CLR alone and in formulation of CLR-gel with excipients. The IR characteristic peaks of CLR were confirmed with the previously reported findings [12]. All the characteristics peaks of CLR and carbopol 934 are at their own position in gel formulation. The IR characteristic peaks of carbopol 934 was similarly found with previously reported finding by Das et al. [31]. The IR peaks of formulation shown that there was no interaction and incompatibility between drug and polymers, suggesting that drug acted same as free drug. Results are shown in Figure 1a, 1b and data is interpreted in Table 3.

Characteristic absorptions peaks at intensity	Functional groups of CLR	Observed peaks
1052 cm^{-1}, 1110 cm^{-1}, 1174 cm^{-1}	(C-O-C stretch)	1052 cm^{-1}, 1110 cm^{-1}, 1174 cm^{-1}
1200-1390 cm^{-1}	(CH2)	1378 cm^{-1}
1425- 1470 cm^{-1}	(N-CH3)	1454.66 cm^{-1}
1680-1690 cm^{-1}	(ketone, C=O)	1691.58 cm^{-1}
1734-1745 cm^{-1}	(lactone, C=O)	1732.2 cm^{-1}
2780-3000 cm^{-1}	(alkane stretching peaks)	2822.2, 2923.57 cm^{-1}
3470-3570 cm^{-1}	(hydrogen bonds between O-H groups)	3487.30 cm^{-1}

Table 3: Characteristic absorptions and functional groups of CLR (BP Specification).

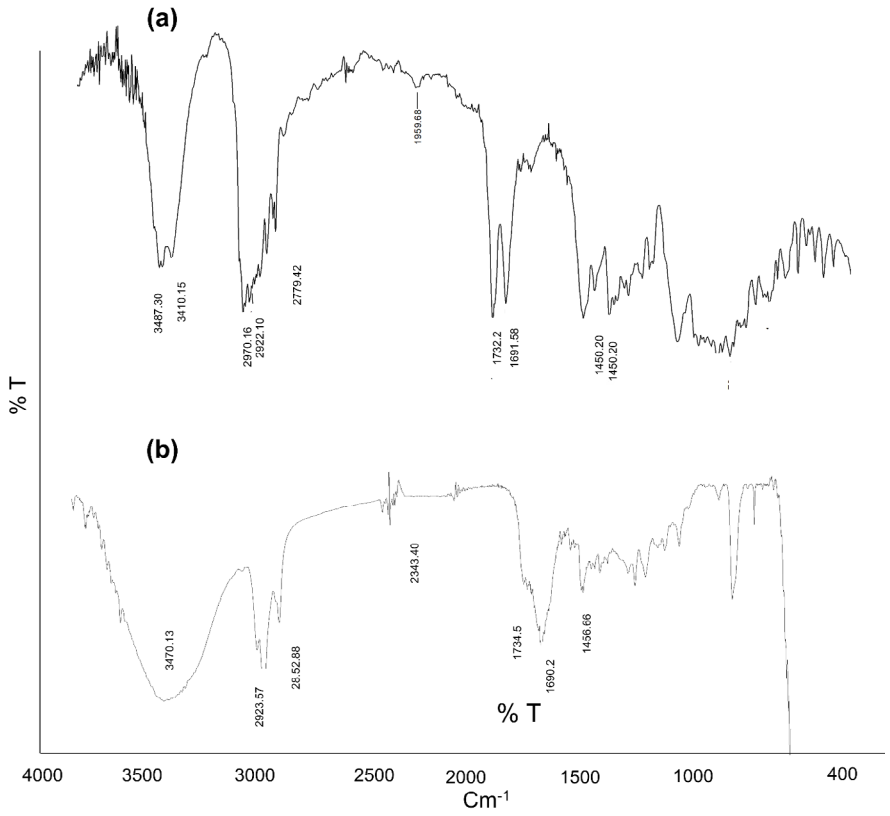

Figure 1(a) and (b): FTIR spectra of: (a) drug (clarithromycin) and (b) clarithromycin and carbopol 934 containing gel.

In-vitro Drug Release

In-vitro release profile of pure CLR and CLR-gel was investigated by dialysis membrane method (DMM). Result from DMM shown that 99.36±2.96 % CLR passed through dialysis membrane within 30 minutes from the solution of pure CLR drug, while 98.19±2.43 % CLR passed within 60 minutes from the CLR-gel, due to presence of polymers in gel which formed compact network like structure. This might be a thick gel barrier, which slows drug release as the diffusional route length for the drug to traverse lengthens [32]. Figure 2 depicts the drug release profile of both pure CLR and CLR-gel. This might be because optimal gel swelling creates a thick gel barrier, which slows drug release as the diffusional route length for the drug to traverse lengthens.

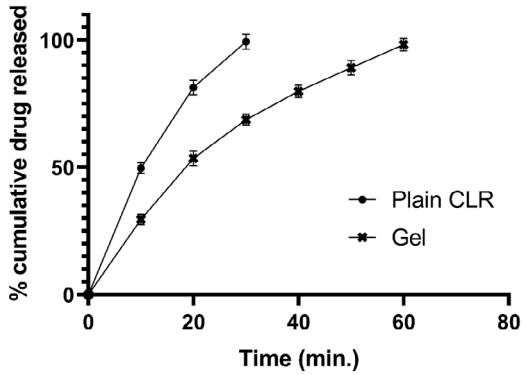

Figure 2: Comparative release profile of formulation F_4 (CLR-gel) to pure CLR.

Stability

5 gm of optimized CLR-gel formulations were packed in aluminium collapsible tubes for stability studies. Stability studies were performed under storage condition at 4±2°C and at room temperature (25±2°C) for duration of 2 months to analyse the effect of different storage temperature on appearance, pH, clarity, viscosity and drug content of formulation after a period of 15, 30, 45 and 60 days. Result of stability studies are presented in Tables 4,5 and Figure 3.

S. No.	Storage period (days)	Visual Appearance		Clarity		pH	
		4±2°C	25±2°C	4±2°C	25±2°C	4±2°C	25±2°C
1	Initial	Transparent	Transparent	Clear	Clear	**6.62±0.13**	**6.62±0.13**
2	15	Transparent	Transparent	Clear	Clear	**6.62±0.13**	**6.62±0.13**
3	30	Transparent	Transparent	Clear	Clear	**6.62±0.13**	**6.62±0.13**
4	45	Transparent	Transparent	Clear	Clear	**6.62±0.13**	**6.62±0.13**
5	60	Transparent	Transparent	Clear	Clear	**6.62±0.13**	**6.62±0.13**

Table 4: Effect of storage temperature on CLR-gel (Appearance, Clarity and pH).

Storage Temperature	% Residual drug content after storage				
	Initial	15 days	30 days	45 days	60 days
4±1°C	97.95±0.55	96.18±1.62	96.24±1.58	96.15±1.56	96.04±1.32
25±1°C	97.95±0.55	96.06±1.62	96.64±1.53	96.34±1.42	96.24±1.39
40±1°C	97.95±0.55	96.04±1.48	96.68±1.48	96.44±1.35	96.64±1.62

Table 5: Effect of Temperature on % drug content.

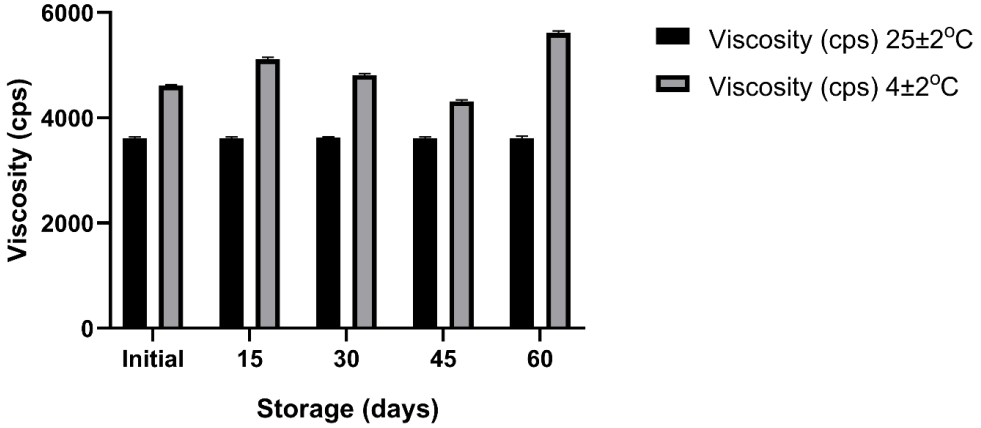

Figure 3: Effect of Temperature on Viscosity of Formulation after 60 days of storage at 4±1°C and 25±1°C.

There were no any changes observes in appearance, pH and clarity at storage temperature, The viscosity of formulation increased at 4±2°C with respect time duration and was not changed at 25±2°C (Figure 3).

The percent residual drug content was calculated to assure the optimum storage condition for the formulation. The optimized gel formulations were filled in screw capped small glass bottles and stored at 4±1°C, 25±1°C and 40±1°C for time period of 15, 30, 45 and 60 days. Data obtained from Table 4 suggests that effect of storage temperature on residual drug content was not significant. The stability study shown that the prepared gel containing CLR has stable for a duration of 60 days at different temperature conditions. Further evaluation of the prepared gel must be conducted at various temperatures and humidity conditions in order to better establish the gel formulation's stability.

Ex-vivo Studies

Anti-microbial Activity: Agar well diffusion method was used to study antimicrobial activity of plane CLR drug solution and CLR-gel formulation against test bacteria (*P. acne*). Different size of zone of inhibition (ZOI) was found in petri plate, zone of inhibition shows the antimicrobial strength of formulation. Where 'C' used as control well with 10 mm diameter, The Zone of inhibition for formulation F_{4b} (CLR-gel bearing 10% lactic acid as an antiscar agent) and F_{4c} (CLR-gel without lactic acid) was found to be 13.97±0.08 mm and 13.83±0.12 mm respectively, while for F_{4a} (CLR drug solution) ZOI was 23.71±0.14 mm. which suggesting that the release of drug from the F_{4b} and F_{4c} formulation was sustained and more effective when compared with the F_{4a} formulation.

The similar results have reported by Jain et al. and confirmed that ZOI of pure CLR has more than CLR-formulations, it might be due to the slow released of CLR from formulations [12].

S. No.	Formulations	ZOI (mean±S.D)
1	F_{4a}	23.71±0.14
2	F_{4b}	13.97±0.08
3	F_{4c}	13.83±0.12

Table 6: Zone of inhibition of formulation.

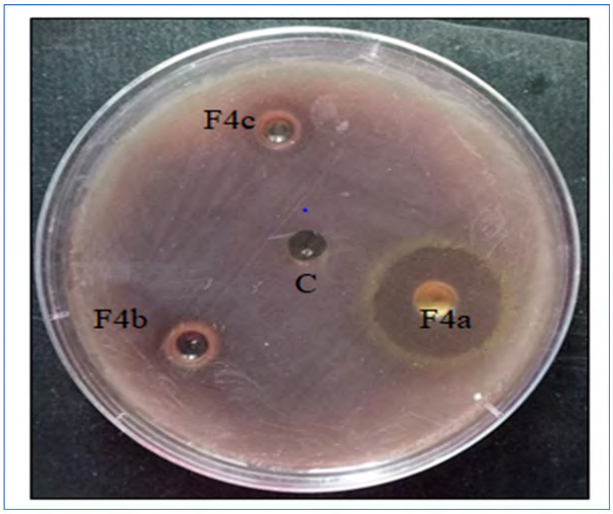

Figure 4: Zone of inhibition of (F4a) CLR drug solution, (F4b) CLR-gel with 10% lactic acid, (F4c) CLR-gel without lactic acid, and (C) as control against the *P. acne.*

Scanning Electron Microscopy (SEM)

Data obtain from SEM images (Figure 5) revealed that antibacterial property of CLR. Sample S_1 was untreated bacterial colony in which bacteria were growing normally, while sample S_2 was treated with CLR-gel, in which morphological perturbation and blebs were found on *P. acnes* surface due to protein synthesis inhibition in bacteria, causing the bactericidal or bacteriostatic effect. This result has been supported by the evidence that the CLR-gel has antibacterial properties in the treatment of acne.

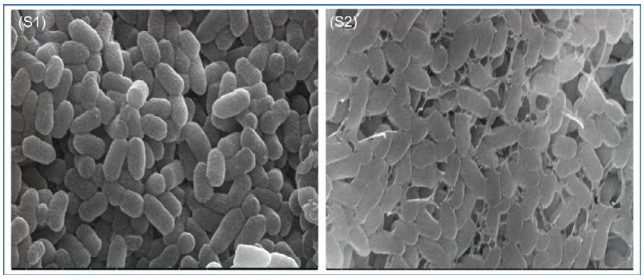

Figure 5: Morphological perturbation and blebs of P. acnes induced by CLR. Image S_1 (untreated *P. acne*), Image S_2 (CLR-gel treated *P. acne*)

Conclusion

Five different formulation of CLR-gel were prepared by using different concentration of carbopol-934 and Propylene Glycol in which formulation F_1, F_2 and F_4 showed acceptable physical properties like pH, spreadability, colour, homogeneity and drug content while formulation F_3 and F_5 showed unacceptable physical properties, among the all formulations F_4 was selected as better formulation on the basis of drug content, percentage drug release and stability at different storage conditions. Therefore, it was concluded that CLR-gel formulation F_4 could be very promising topical drug delivery system for the treatment of acne and other skin infections.

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Conflict of Interest

All authors (V. Gour, A. Kumari, S. Gound, R. Mondal, D. Jain, R. Roy, V. Soni) declare that they have no conflict of interest.

References

1. Zouboulis CC (2004) Acne and sebaceous gland function. Clinics in dermatology 22(5): 360-366.

2. Kanwar IL, Haider T, Kumari A, Dubey S, Jain P, et al.

(2018) Models for acne: A comprehensive study. Drug Discov Ther 12(6): 329-340.

3. Thiboutot D, Gollnick H, Bettoli V, Dréno B, Kang S, et al. (2009) New insights into the management of acne: an update from the Global Alliance to Improve Outcomes in Acne group. Journal of the American Academy of Dermatology 60(5): S1-S50.

4. Hazarika N (2020) Acne vulgaris: new evidence in pathogenesis and future modalities of treatment. J Dermatolog Treat 32(3): 277-285.

5. Kaneko N, Kurata M, Yamamoto T, Morikawa S, Masumoto J (2019) The role of interleukin-1 in general pathology. Inflammation and Regeneration 39(1): 12.

6. Karadag AS, Kayiran MA, Wu CY, Chen W, Parish LC (2021) Antibiotic resistance in acne: changes, consequences and concerns. J Eur Acad Dermatol Venereol 35(1): 73-78.

7. Aubin GG, Portillo ME, Trampuz A, Corvec S (2014) Propionibacterium acnes, an emerging pathogen: from acne to implant-infections, from phylotype to resistance. Med Mal Infect 44(6): 241-250.

8. Bellini C (2012) Cited or read?. Lancet 379(9813): 314.

9. Kameswararao K, Sujani C, Koteswararao N, Rajarao A, Satyanarayanamma PNS (2019) A Brief Review on Acne Vulgaris. Research Journal of Pharmacology and Pharmacodynamics 11(3): 109-119.

10. Singh N, Singh A, Pandey K, Nimisha (2020) Current insights for the management of acne in the modern era. Recent Pat Antiinfect Drug Discov 15(1): 3-29.

11. Tan AU, Schlosser BJ, Paller AS (2018) A review of diagnosis and treatment of acne in adult female patients. International journal of women's dermatology 4(2): 56-71.

12. Jain SK, Haider T, Kumar A, Jain A (2016) Lectin-Conjugated Clarithromycin and Acetohydroxamic Acid-Loaded PLGA Nanoparticles: a Novel Approach for Effective Treatment of H. pylori. AAPS PharmSciTech 17(5): 1131-1140.

13. Zuckerman JM, Qamar F, Bono BR (2011) Review of macrolides (azithromycin, clarithromycin), ketolids (telithromycin) and glycylcyclines (tigecycline). Med Clin North Am 95(4): 761-791.

14. Van ES, Yu R (1989) Alpha hydroxy acids: procedures for use in clinical practice. Cutis 43(3): 222-228.

15. Nautiyal A, Wairkar S (2021) Management of hyperpigmentation: Current treatments and emerging therapies. Pigment Cell Melanoma Res 34(6): 1000-1014.

16. Sharquie KE, Abdulla MS (2005) Treatment of vitiligo with topical 15% lactic acid solution in combination with ultra violet-A. Saudi Med J 26(12): 2011.

17. Rendl M, Mayer C, Weninger W, Tschachler E (2001) Topically applied lactic acid increases spontaneous secretion of vascular endothelial growth factor by human reconstructed epidermis. The British journal of dermatology 145(1): 3-9.

18. Brozovic S, Vucicevic-Boras V, Mravak-Stipetic M, Jukic S, Kleinheinz J, et al. (2002) Salivary levels of vascular endothelial growth factor (VEGF) in recurrent aphthous ulceration. Journal of oral pathology & medicine 31(2): 106-108.

19. Kelidari HR, Saeedi M, Hajheydari Z, Akbari J, Morteza-Semnani K, et al. (2016) Spironolactone loaded nanostructured lipid carrier gel for effective treatment of mild and moderate acne vulgaris: A randomized, double-blind, prospective trial. Colloids Surf B Biointerfaces 146: 47-53.

20. Shivhare U, Jain K, Mathur V, Bhusari KP, Roy AA (2009) Formulation Development and Evaluation of Diclofenac Sodium Gel using Water Soluble Polyacrylamide Polymer. Digest Journal of Nanomaterials & Biostructures (DJNB) 4(2): 285-290.

21. Bachhav YG, Patravale VB (2010) Formulation of meloxicam gel for topical application: In vitro and in vivo evaluation. Acta pharmaceutica 60(2): 153-163.

22. Shingade G (2012) Development of nabumetone topical gels: effect of formulation variables on the release of nabumetone. World J Pharm Research 1(3): 776-785.

23. Setty C, Babubhai S, Pathan I (2010) Development of valdecoxib topical gels: effect of formulation variables on the release of valdecoxib. Int J Pharm Pharma Sci 2(1): 70-73.

24. Raut S, Uplanchiwar V, Gahane A, Santosh B, Shrishail P, et al. (2012) Development, characterization and investigation of anti-inflammatory potential of valdecoxib topical gels. NISCAIR-CSIR.

25. Tanwar Y, Jain AK (2012) Formulation and evaluation of topical diclofenac sodium gel using different gelling agent. Asian Journal of Pharmaceutical Research and Health Care 4(1).

26. Han SM, Lee KG, Pak SC (2013) Effects of cosmetics

containing purified honeybee (Apis mellifera L.) venom on acne vulgaris. J Integr Med 11(5): 320-326.

27. Tan YT, Peh KK, Al-Hanbali O (2000) Effect of Carbopol and polyvinylpyrrolidone on the mechanical, rheological, and release properties of bioadhesive polyethylene glycol gels. AAPS PharmSciTech 1(3): E24.

28. Zheng Y, Ouyang WQ, Wei YP (2016) Effects of Carbopol(®) 934 proportion on nanoemulsion gel for topical and transdermal drug delivery: a skin permeation study. Int J Nanomedicine 11: 5971-5987.

29. Khan AW, Kotta S, Ansari SH, Sharma RK, Kumar A, et al. (2013) Formulation development, optimization and evaluation of aloe vera gel for wound healing. Pharmacogn Mag 9(1): S6-S10.

30. Kim JY, Song JY, Lee EJ, Park Sk (2003) Rheological properties and microstructures of Carbopol gel network system. Colloid and Polymer Science 281(7): 614-623.

31. Das T, Manna M, Rudra A (2020) Formulation and characterization of papaya leaf gel. GSC Biological and Pharmaceutical Sciences 10(3): 89-94.

32. Jain P, Taleuzzaman M, Kala C, Gupta DK, Ali A, et al. (2021) Quality by design (Qbd) assisted development of phytosomal gel of aloe vera extract for topical delivery. Journal of Liposome Research 31(4): 381-388.