Novel Formulation and Clinical Evaluation of Levofloxacin Hemihydrategel for the Treatment of Impetigo

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Abstract: Levofloxacin hemihydrate (LVFX) is a fluoroquinolone antibiotic used for the treatment of complicated and uncomplicated skin infections. Impetigo is a highly infectious superficial bacterial disease, most common among pre-school children. The present study was designed to formulate and evaluate topical gel containing levofloxacin hemihydrate for treatment of impetigo. The gel was formulated using different types and concentrations of gelling polymers. The used polymers, viz; hydroxypropylmethyl cellulose (HPMC), sodium carboxymethyl cellulose (NaCMC), carbopol 934, sodium alginate (Na-alginate), pluronic ® F-127 and poly vinyl alchohol (PVA 14000). Drug-polymers compatibility studies were carried out using DSC and FT-IR techniques, then the prepared formulae were characterized physically in terms of pH, drug content, spread ability and rheological properties. Drug-polymers compatibility studies were carried out using DSC and FT-IR techniques. In-vitro drug release in phosphate buffer pH 7.4 and kinetics of the drug release were studied. In vitro microbiological studies of (LVFX) gel were performed using agar cup diffusion method. Patients with clinically diagnosed impetigo were topically treated with the best formula of LVFX gel. Results have revealed that the used polymers are compatible with the drug. The prepared LVFX gels with different gelling agents showed acceptable physical properties and good drug release. Among all the prepared gels, formula (G1) using HPMC as a gelling agent attained superior physical properties, drug release (80.30±0.11%) after 2 hrs. No significant changes in the physical properties and in the percent of drug release were observed for formula (G1) at (5 ±2°C/60 ±5 % RH, 25±2°C / 65 ±5% RH and 40±2°C / 75 ± 5 RH) after 3 months of storage. These results we reconfirmed by thin layer chromatography. Also, formula (G1) was found to have the highest antimicrobial activity against methicillin resistant staphylococcus aureus (MRSA), streptococcus pyogenes, Escherichia coli, and Klebsiellapneumoniae. LVFX topical gel (G1) was well tolerated with high rates of clinical response (significant reduction in the time of healing after 4 days) for treating impetigo.

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1. Introduction

Topical delivery systems are an attractive route for local and systemic treatments. The delivery of drugs onto the skin is recognized as an effective mean of therapy for local dermatologic diseases. Formulated drugs may penetrate deep into the skin and hence give good absorption[1].

Impetigo is a highly contagious bacterial skin infection (pyoderma) affecting children worldwide which caused by Staphylococcus, Streptococcus or MRSA [2]. However, impetigo caused by MRSA can predispose a patient to a deeper and more serious infection[3].

Levofloxacin hemihydrate is used as anti-microbial agent for the treatment of variety of infectious diseases. Unlike earlier fluoroquinolones, levofloxacin is active against gram negative as well as gram positive bacteria. It is used in the treatment of bronchitis, urinary tractinfections, pneumonia, skin and soft tissues infections[4]. Levofloxacin hemihydrate is available in the market in the form of oral tablets, injections and eye drops[5].

From the previous studies, there are no researches dealing with topical formulations of LVFX gel. Topical application is less likely to cause side effects compared to systemic therapy. However, the emergence of resistant microorganisms had led to failure in the treatment of different skin diseases by the previous available antimicrobial agents.

In this study, suggested formulation of LVFX topical gels were developed for treatment of impetigo. Also, the high local drug concentration in topical application will be able to overwhelm many mutation resistances. Therefore, LVFX (1%w/w) was

formulated into gels using certain types and concentrations of gelling agents such as; hydroxypropylmethyl cellulose (HPMC), sodium carboxymethyl cellulose (NaCMC), carbopol 934, sodium alginate (Na-alginate), pluronic® F-127 andpoly vinyl alchohol (PVA 14000). Compatibility studies, physical characterizations of the drug with the used polymers, in vitro drug release of the drug and its antibacterial activity were evaluated. Then the best selected formula was further investigated for stability studies and clinical efficacy in patients with impetigo.

2. Experimental

Materials

Levofloxacin hemihydrate was kindly provided by Amoun Pharmaceutical Company, Cairo, Egypt, Sodium carboxymethyl cellulose (NaCMC) (Adwic, EL-Nasr Pharmaceutical Chemicals Co., Egypt), Hydroxypropylmethyl cellulose (HPMC) (Aldrich Chem. Co., USA), Sodium alginate (Na-alginate) (Judex Laboratories Reagent, UK), Pluronic® F-127 (Sigma Chemical Co., USA) and Carbopol 934(C.P. Evan Co., England). All the other chemicals were of analytical grade. Standard cellophane membranes (molecular cut of range equals 12000) (Sigma Chem. Co., USA).

Methods

Compatibility studies:

Differential scanning calorimetry (DSC) studies:

Thermal characterizations of pure drug and its physical mixtures (1% w/w) with the used polymers were performed using Differential Scanning Calorimeter (DSC– 50, Schimadzu model, Seisakusho Ltd., Kyoto, Japan). The instrument was calibrated with pure indium. The thermograms were obtained by heating (5 mg) of the drug alone and its physical mixture encapsulated in flat bottom aluminium pan at a scanning rate of 10^{o}C/min. from 30^{o}C – 300^{o}C under nitrogen gas stream at a flow rate of 40 ml/min. An empty pan was used as a reference and subjected to the same treatments[6].

Fourier Transform Infrared (FT-IR) Studies:

About 2-3 mg of the samples were mixed with potassium bromide then compressed into a disc at 4-ton pressure. IR absorption spectra were recorded using a FT-IR (Spectrophotometer Nicolet 6700, Japan) over a range of 200-4000 cm^{-1}. Data management was performed using Omnic software[7].

Preparation of the levofloxacin hemihydrate gels:

The composition of the formulated gels of LVFX is illustrated in Table (1). The required amount of the drug (1% w/w) was dissolved in 100 ml phosphate buffer solution of PH7.4 using magnetic stirrer (Human Lab. co., HS-18, Korea), then the required weight of each polymer was added slowly with continuous stirring until translucent gels were formed. Care was taken that no lumps of the polymer were formed during stirring[8].

Carbopol 934 was dispersed in water and homogenized by magnetic stirrer for 30 min then left to equilibrate for 24 h. Then the pH was adjusted to 5-7 using triethanolamine[9]. Pluronic F 127 gels were prepared using the cold method, the polymer was added slowly to cold water with continuous agitation. The mixture was then stored overnight at 4° C. Aqueous Pluronic dispersions are solution at low temperature and they are converted to semisolid gel at room temperature[10].

Evaluation of Levofloxacin hemihydrate gels:

Organoleptic properties:

The appearance was checked visually for color, homogeneity and transparency. They were tested for their appearance and presence of anylumps[11].

Drug content determination:

Drug content was determined by dissolving accurately weighed 1 g of the gel formulation in phosphate buffer solution of pH 7.4 using magnetic stirrer in order to get complete solubility of the drug. The mixture was then quantitatively transferred into 25 ml volumetric flask and completed with phosphate buffer solution. The absorbance of the drug was recorded using UV Spectrophotometer (UV-Visible Spectrophotometer, Jenway-model6305, England) at 293 nm and the percentage of drug content was calculated in each gel preparation[12].

PH measurements:

A digital pH meter, (Jenway-model 3310, England) was used to determine the pH of the formulae (G1-G12). The results are the mean of three determinations[13].

Spreadability:

Sample of 0.25 g of each gel formula was placed in between two glass slides of specific length and left for 5 minutes to ensure no more spreading. The upper slide was fitted with a string that was tied with a fixed weight. The string was passed over a pulley, and the weight was hung from the string. Under the weight, the upper glass slide took time to slip off and the time was noted. The less the time taken for separation of two slides, the better the spread ability[10].

Rheological studies:

The rheological behavior of the prepared formulae was determined using Brookfield Viscometer (Brookfield Engineering Laboratories, Inc., USA), using spindle number 95 at temperature 25°C. Thespindle was kept to rotate for one minute before measuring the shear stress and viscosity. The viscosity of the formulation was determined at different speed conditions (10-100 rpm). The test was done intriplicates and the mean was calculated[14].

In vitro release of LVFX gels:

In vitrorelease of LVFX from the prepared gels was studied using dialysis method[15]. Sample of each formula (0.5 g) was accurately weighed and placed on a semi permeable cellophane membrane (previously immersed in phosphate buffer ofp H 7.4 for 24 hours) to occupy a circle of 2.5 cm diameter and surface area 4.9 cm^2. The loaded membrane (donor compartment) was firmly stretched over the lower open end of a glass tube of 2.5 cm diameter and made water tight by rubber band. The tube was then immersed in a beaker (receiver compartment) containing 100 ml of the release medium of phosphate buffer (pH 7.4). The system was maintained for 2 hours at 37 ± 0.5°C in a thermostatic shaker water bath (Dihan Scientific, WSB 45, Korea) at 50 rpm. Samples of 5 ml were withdrawn from the receiver at intervals of 0.25, 0.5, 1, 1.5 and 2 hrs.[15, 16]. The volume of each sample was replaced by the same volume of fresh buffer solution (kept at the same temperature) to maintain constant volume. Samples were analyzed for LVFX content spectrophotometrically at λ max 293 nm against blank similarly treated. All experiments were carried out in triplicate and the average values were calculated[15, 16].

Kinetic analysis of the in vitro release data of the drug:

To analyze the mechanism of drug release from the prepared gels, the results of in-vitro releasedata were fitted to various kinetic equations; zero order, first order and Higuchi diffusion model[17-19].

Stability study:

The stability study was performed as International Conference Harmonisation guidelines (ICH)[20]. The best selected formula (G1) was filled in the collapsible tubes and stored at certain temperatures and humidity conditions (5 ±2°C/60 ±5 % RH, 25±2°C / 65 ±5% RH and 40±2°C / 75 ± 5 RH) for a period of 3 months[21]. Visual inspection, drug content and pH were evaluated monthly. Thin layer chromatography (TLC, Camag, Switzerland, Germany) technique was used to study the stability of LVFXgel[22]. Levofloxacin hemihydrate gel (0.1 g) was extracted from stored formula at the end of storage (3 months) using 8 mlof methanol and sonicated for 30 min. with heating at 50°C. Then, the volume was adjusted with methanol to 10 ml. Two microliter volume of solution was spotted on TLC plats as well as 2 µl. of freshly prepared sock solution of LVFX. Chromatograms were developed using butanol: methanol: 25% ammonia (5:1:1.5 v/v/v)[23]. The plats were dried at room temperature (25 °C). The TLC plate scanner III in the reflection-absorbance mode at 293 nm for all measurements and operated by WINCATS software, version 1.4.4.6337. The calculation were performed depending on the peak area of LVFX[24].

Antibacterial studies:

The antibacterial activity of the selected formula of LVFXgel (G1) against MRSA, streptococcus pyogenes, Escherichia coli, and Klebsiellapneumoniae were evaluated by agar cup diffusion method[25]. A layer of nutrient agar (20ml) seeded withthe tested micro-organisms (0.2 ml) were allowed to solidify in the petri plate. Pores were made in the solidified agar layer with the help of sterile borer at4mm diameter. Then accurate amount of the prepared gels were taken by syringe poured in each pore. The plates were incubated in an oven for 24 hours at 37°C. The zone of inhibition (in mm) was measured using zone reader and the results are the mean of three determinations [26].

Clinical evaluation of the selected LVFX formula:

Formula (G1) of LVFX using HPMC gel base (showed the highest in vitro release of the drug and good stability and good antibacterial activity) was chosen for further clinical application in treatment of impetigo patients.

Patients:

The protocol was approved by the medical ethics committee of the faculty of medicine, Assiut University. The clinical study was conducted on 30patients at the Dermatology and Venereology and Andrology Department, Assiut University hospital. Ten male and twenty female patients were involved in the clinical study, the age of whom ranged from 2 to 14 years old. The diagnosis of impetigo was mainly a clinical one, but further confirmation was done by microbiological investigations of skin swap. For all patients, collection of data was done including personal information like name, age, gender, address, medication history and history of present illness. None of the selected patients received any other treatment of impetigo either topical or systematic.

Clinical study design:

A randomized, placebo controlled, double blind study was performed. The exclusion criteria from the test were hypersensitivity to LVFX, patients who had received a topical or systemic antibiotic within the preceding 72 hours, patients on whom the total surface area of the lesion exceeded 50 cm^2, and those with an infection required systemic antibiotic treatment. The patients were divided into three groups; as follows, group I: patients received placebo gel, group II: patients received 1 % w/w LVFX gel (G1) and group III: patients received marketed product (Fucidine cream, Leopharmaceuticals, New Cairo Egypt).

Clinical response to treatment:

All patients were instructed to apply LVFX gel twice daily in the morning and in the evening for 7-

14 days. An applicator was provided and patients were taught to use it to homogenously distribute the drug in the base on application. Follow up and close supervision were done whenever possible during the treatment course in order to assess the signs of healing.

Clinical efficacy:

Efficacy of the prepared gels and Fucidine cream was assessed by clinical investigations of the patients, counts of lesions and crusts, and microbiological isolation and identification of bacteria at baseline and at the end of the study (7-14 days). Colored photographs were taken for every patient before and after treatment in order to assess the degree of response to the therapy. Clinical outcome in patients of group (II) received medicated LVFX gel and group III receiving Fucidine cream was scored as excellent, good or poor compared with patients received placebo gel (group I).

Adverse events:

Patients were monitored for any adverse effects during treatment course. Any problem in drug application was also reported in order to ensure the highest degree of patient comfort and compliance.

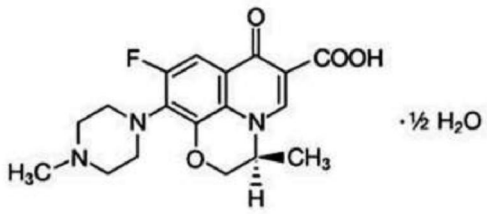
Statistically analysis:

Experimental results were expressed as mean ± SD. Student's t-test and two-way analysis of variance (ANOVA) were applied to check significant differences in the obtained results. Differences were considered to be statistically significant at $p < 0.05$.

3. Results and discussion

Differential scanning calorimetry (DSC) studies:

The DSC thermo grams of the drug alone and itsphysical mixtures with the used polymers (1:1 w/w) are shown in Fig. (1). Levofloxacin hemihydrates show a sharp endothermic peak around 228 ºC corresponding to its melting point[27]. The thermograms of the physical mixture of the drug with the used polymers show the same endothermic peak of the drug at 228 ºC with a decrease in their intensity which may be attributed to the dilution effect. From the obtained results, it is revealed that the drug is compatible with the used polymers in the prepared gels.



Fourier Transform Infrared (FT-IR) Studies:

Fig. (2) shows the IR spectra of LVFX alone and its physical mixtures with the investigated polymers (1:1 w/w). Infra-red spectrum of pure drug show a major characteristic bands at 3265 cm^{-1} due to carboxylic group stretching, at 2931 cm^{-1} due to alkanes group stretching, at 1724 cm^{-1}due to stretching of carbonyl group, at 1294 cm^{-1} due to stretching of amines and at 1100 - 1400 $cm^{-1}$and due to the presence of halogen group[27, 28]. The principal peaks of LVFX were observed in the spectra of its physical mixtures with the used polymers [25].

Organoleptic properties:

All the prepared formulae are transparent and homogenous as illustrated in Table (2). The color of the freshly prepared LVFX gels is light yellow. While, yellowish color of the gels with Na alginate and PVA was observed.

Drug content determination:

The drug content of different LVFX topical gels were found to range from 97.2±0.12 to 99.8±0.13 % w/w as shown in Table (2). The higher percent of the drug content indicates that the drug is uniformly distributed in the certain prepared formulae.

PH measurements:

Also, it was found that the pH of the prepared formulae were found to be in the range of6.4±0.13 to 7.4±0.14.

Spreadability:

Table (3) illustrates that all the prepared formulae of LVFX gels gave a relatively goods readability. The obtained results were in concomitant with their viscosities.

Rheological studies:

Fig. (3) shows that the concavity of the curves of the prepared LVFX gels toward the shear rate axis, indicating that the prepared gels exhibited Non-Newtonian (shear thinning) pseudo-plastic flow. These results are in agreement with *Nayak, et.al., (2012)*, who have reported that moxifloxacin hydrochloride ophthalmic gel has pseudo-plastic flow when using Carbopol 934 and HPMC as a gelling agent[29].

In vitro release of LVFX from the prepared gels:

Figs. (4-6) show the release profiles of LVFX (1% w/w) from the prepared gels using certain types and concentrations of polymers as gelling agents in pH 7.4 for 2 hrs. The used polymers are namely; hydroxypropylmethyl cellulose (HPMC), sodium carboxymethyl cellulose (NaCMC), carbopol 934, sodium alginate (Na-alginate), pluronic® F-127 andpoly vinyl alchohol (PVA 14000). From the obtained results, it is observed that increasing the concentration of the used polymers led to decrease in the release rate of LVFX from the prepared gels. This can be explained by increasing the viscosity of the gel bases. The percent released of LVFX (G1-G4) from

the prepared gels using different concentrations of HPMC and NaCMC as gelling agents were ranged from 30.48±0.01% to 80.30±0.11%. Regarding the gel type the percent released of the drug from the prepared gels decreased in the following order: HPMC (2%w/w) > HPMC (6%w/w) > NaCMC (3%w/w) > NaCMC (6%w/w), as shown in Fig. (4). Formula (G1) using HPMC (2%w/w) as a gelling agent show the maximum percentage of the drug release (80.30±0.11%) at 2 hrs. While, formula (G4) containing NaCMC (6%w/w) has the lowest percent released of the drug (30.48±0.01%) among all the investigated formulae after the same time. A higher significant difference ($P<0.05$) was observed between the release rate of LVFX from HPMC and NaCMC gel bases. The higher release rate of LVFX from HPMC than that from NaCMC as gelling agent may be attributed to the higher solubility of LVFX in NaCMC, in addition to the higher viscosity of NaCMC[30]. This results coincided with the obtained by Samy et.al[31]. Who have concluded that the release rate of moxifloxacin from the prepared gels using HPMC was higher than that from the gel prepared using NaCMC as gel bases [31].

Fig. (5) shows the release profiles of LVFX (1%w/w) from the prepared gel formulae (G5-G8) at pH 7.4 using Carbopol 934 (0.5 & 1%w/w) and Na alginate (5 & 8%w/w) as gel bases respectively. The release rate of the drug from these polymers is decreasing in the following order; Carbopol (0.5%w/w) > Carbopol (1%w/w)> Na alginate (5%w/w) > Na alginate (8%w/w)[13, 31]. The enhanced percent of the drug release (76.71 ± 0.04) from Carbopolgel base may be attributed to the presence of pores in the gel which allow relatively free release of the drug to the vehicle and lack of over solubilisation of the lipophilic drug in aqueous vehicle and hence readily available for release[26]. While, the percent release of LVFX (32.3±80.15) from Carbopol 1%w/w was observed after the same time. The drug release has significantly decreased ($P<0.05$) upon increasing the concentration of Carbopol from 0.5% to 1 %w/w.

The release rate of LVFX from the prepared formulae (G9-G12) using pluronic 127 (20 & 25 %w/w) and PVA (15 & 20 %w/w) respectively as a gelling agents at pH 7.4 for 2 hrs. are illustrated in Fig. (6). The release rate of LVFX from gels using pluronic 127 was higher than that from gels using PVA as gel bases. This may be due to the formation of monomolecular micelles and low viscosity of pluronic 127 compared with PVA[13].

Analysis of the in vitro release data:

The kinetic analysis of release data of LVFX from the prepared gels is illustrated in Table (4). In vitro drug release study was fitted to various kinetics equations like zero order, first order and Higuchi diffusion model. The release rates of LVFX from the prepared gels are best fitted to simplified Higuchi equation which illustrated by the highest correlation coefficient (r^2). This means that the release of LVFX from this formulae depend on diffusion mechanism. While formulae G5, G6, G11 and G12 followed zero-order kinetics that may be attributed to the high viscosity of the prepared gels.

Stability Study:

Tables (5-6) show the stability study of the best selected LVFX gel formula (G1) stored under certain conditions (5 ±2°C/60 ±5 % RH, 25±2°C / 65 ±5% RH and 40±2°C / 75 ± 5 RH) for a period of 3 months. There were no significant changes with respect to organoleptic, physical properties and in-vitro drug release under ambient conditions at 25±2ºC/65±5% RH and refrigeration at 5±2ºC/60±5%RH. No significant change in pH or drug content of stored formula when checked monthly.

Fig. (7) shows that there is no significant difference between RF values of LVFX extracted from stored formula (G1) was the same as that of LVFX stock solution (0.45 mm) confirming that the drug is stable in the selected formula under the investigated storage conditions. Therefore, this formula (G1) was strongly recommended for further clinical application on patients with impetigo.

Antibacterial studies:

The best gel formula (G1) which gave the highestin vitro drug release, acceptable rheological behaviour and good stability was selected for antibacterial activity against the tested microorganisms. The inhibition zone of formula (G1) against MRSA, S. pyogenes, *E. coli*, and K.pneumoniaewere40±0.43, 34±0.22 and 26±0.23 and 23±0.15 mm respectively. These results indicate the efficiency of the prepared gel of LVFX against MRSA as illustrated in Fig. (8).

Clinical application of the gels in patients with impetigo:

A total of 30 patients with clinically diagnosed impetigo were randomized to one dose group of 1% LVFX gel (G1) in the study. Twenty eight patients (93%) had microbiologically confirmed infection. Treatments were topically applied using LVFX gel (G1) administered two times a day (TID, in the morning and in the evening) for 14 days, to all randomized subjects. The results of treatment are demonstrated in Table (6). These results could be statistically analysed according to the time of healing (days) after topical application of LVFX gel formula. The analysis using t- tests were adopted when data were normally distributed (SPSS, Chicago, IL). It is worth mentioning, that the tested formula didn't show

any adverse effects on any patients. In addition, the short period of treatment and the ease of application had increased patient compliance and their adherence to treatment. A total of 28 subjects (93%) complete treatment for mild and sever cases of impetigo patients received LVFX gel (G1) in the period ranged from 4 to 7 days and they were assessed by clinical investigations of the patients, counts of lesions and crusts at the end of treatment (Table 6). While, topical application of Fucidine cream (marketed product) for mild and sever cases of impetigo patients (group III) were taken (9-13) days for complete healing. There was a significant difference ($P<0.05$) in the reduction of healing time between patient treated with LVFX (G1) and the marketed product (Fucidine cream).

Colored photographs (Figs.9 & 10) were taken for patient before and after treatment with LVFX gel in order to assess the degree of response to the therapy. Fig (9) showscomplete cure and absolute disappearance of crusts after 5 days of treatment with topical LVFX gel (G1) for child who has ordinary bullous impetigo. Good improvement was observed after 4 days of treatment with LVFX gel for male child suffering from purulent crusts of scalp impetigo, as shown in Fig. (10).

LVFX topical gel administration was well tolerated with high rates of clinical and microbiological responses for treating impetigo. The goal is to make available and global treatment for impetigo and other superficial infections by the prepared gel formulations.

Table 1: Composition of prepared LVFX gel formulations:

Composition	G1	G2	G3	G4	G5	G6	G7	G8	G9	G 10	G11	G12
LEVX	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
HPMC	2	6	---	---	---	---	---	---	---	---	---	---
Na CMC	---	---	3	6	---	---	---	---	---	---	---	---
Carbopol 934	---	---	---	---	0.5	1	---	---	---	---	---	---
Na alginate	---	---	---	---	---	---	5	8	---	---	---	---
Pluronic 127	---	---	---	---	---	---	---	---	20	25	---	---
PVA 14000	---	---	---	---	---	---	---	---	---	---	15	20
Water to	100	100	100	100	100	100	100	100	100	100	100	100

Table 2: organoleptic properties, drug content and pH of the prepared LVFX gel formulae:

Formula No.	pH value	Drug content	Colour	Homogeneity	Transparency
G1	6.8± 0.10	99.5± 0.19	Light yellow	All gel formulae were homogenous	All gel formulae are translucent
G2	6.4± 0.13	99.8± 0.13			
G3	7.3± 0.12	97.2± 0.12			
G4	7.4± 0.14	98.8± 0.15			
G5	6.7± 0.13	97.5± 0.12			
G6	6.5± 0.12	97.8± 0.15			
G7	6.7± 0.11	98.4± 0.19	yellowish		
G8	7.2± 0.13	99.6± 0.13			
G9	7.3± 0.11	99.5± 0.17	Light yellow		
G10	7.2± 0.13	99.6± 0.13			
G11	6.9± 0.12	98.9± 0.11	yellowish		
G12	6.8± 0.11	99.5± 0.19			

Table 3: Spreadability of the prepared LVFX gel formulae:

Formula No.	Polymer	Spreaded circle diameter Average (cm) ± SD
G1	2% HPMC	3.14 ± 0.03
G2	6% HPMC	2.15± 0.01
G3	3% Na CMC	2.05± 0.01
G4	6% Na CMC	1.12± 0.02
G5	0.5% Carb.934	3.04 ± 0.10
G6	1% Carb.934	1.15± 0.06
G7	5%Na alginate	2.10± 0.08
G8	8% Na alginate	1.50± 0.09
G9	25% P-127	2.00± 0.03
G10	20% P-127	2.70± 0.07
G11	15% PVA 14000	2.98± 0.05
G12	20% PVA 14000	2.00± 0.09

Table 4: Kinetic analysis of the release data of LVFX from the prepared gels

Formulae no.	Zero Order		First Order		Higuchi Diffusion model		Best fitted model
	R	K_0	r	K_f	r	K_h	
G1	0.9898	0.2105	0.9904	0.0033	0.9961	3.8668	
G2	0.9966	0.1994	0.9972	0.0030	0.9993	3.6460	
G3	0.9952	0.3711	0.9963	0.0075	0.9976	6.6700	
G4	0.9936	0.3425	0.9956	0.0064	0.9979	6.1474	Higuchi Diffusion model
G5	0.9990	0.3248	0.9987	0.0057	0.9926	5.7996	
G6	0.9987	0.3046	0.9973	0.0051	0.9966	5.3859	Zero Order
G7	0.9927	0.0551	0.9940	0.0005	0.9959	0.9852	
G8	0.9960	0.0480	0.9981	0.0006	0.9999	0.5885	
G9	0.9924	0.1869	0.9916	0.0028	0.9948	3.4026	
G10	0.9962	0.1807	0.9970	0.0029	0.9982	3.2902	Higuchi Diffusion model
G11	0.9963	0.4530	0.9933	0.0006	0.9949	4.0019	
G12	0.9986	0.2484	0.9921	0.0005	0.9960	3.8681	Zero Order

Where **r**: correlation coefficient, K_0: zero order rate constant (µg. min^{-1}), K_f: first order rate constant (min^{-1}) and k_h: Higuchi rate constant (µg. $min^{-1/2}$).

Table 5: Physical properties and release study of LVFX gel formula (G1) using 2% w/w HPMC as gelling base under different conditions of temperature and humidity during three months of storage.

Time of storage (months)		Physical appearance	color	Homogeneity	Texture	pH	Drug content (%)	Viscosity (CP)
Fresh Formula	**0**	Translucent	Pale yellow	Acceptable	smooth	6.8±0.10	99.5±0.19	20340
5 ± 2^{0}C/60 ±5 % RH	**1**	Translucent	Pale yellow	Acceptable	smooth	6.6±0.12	99.4±0.14	20340
	2					6.4±0.15	98.99±0.12	20463
	3					6.3±0.10	97.12±0.17	20570
25±2^{0}C / 65 ±5% RH	**1**	Translucent	Pale yellow	Acceptable	smooth	6.5±0.02	98.80±0.11	20340
	2					6.4±0.05	97.99±0.17	20430
	3					6.2±0.09	97.34±0.19	20645
40±2^{0}C / 75 ± 5 % RH	**1**	Transparent	yellow	good	smooth	6.4±0.12	100.1±0.15	20450
	2	Cloudy	Dark yellow	Not good	Slightly rough	6.2±0.14	102.2±0.16	20500
	3			Not good	Slightly rough	6.00±0.15	101.3±0.12	20700

Table 6: Cumulative percent release of LVFX from stored gel (G1) after three months storage under different temperatures and humidities:

Time (min.)	% of LVFX released from the stored gel (G3)			
	Fresh (G3) At 0 day	5±2^{0}C/60±5 % RH	25±2^{0}C/65 ±5% RH	40±2^{0}C/75 ± 5 % RH
0	0.00	0.00	0.00	0.00
15	27.66± 0.23	25.22 ± 0.25	27.12 ± 0.15	24.12 ± 0.15
30	39.64± 0.50	33.34± 0.15	35.32 ± 0.25	32.37 ± 0.14
60	60.63± 0.42	55.43± 0.12	59.23± 0.15	50.23± 0.19
90	65.92± 0.23	60.22 ± 0.25	62.27 ± 0.25	58.12 ± 0.15
120	80.30± 0.11	77.20 ± 0.14	79.29 ± 0.19	75.25 ± 0.15

Table 6: Clinical assessment of patients with impetigo treated with LVFXgel formula (G1).

Formulae	Patient			Duration of treatment (days)	Outcome
	Age (years)	Gender	Impetigo type		
Group I Placebo LVFX gel (G1)	10	Male	Moderate	>14	No improvement
	9	Male	Mild	>14	No improvement
	8	Female	Moderate	>14	No improvement
	5	Female	Mild	>14	No improvement
	12	Female	Mild	>14	No improvement
Group II Medicated LVFX gel	9	Female	Mild	7	Good
	10	Male	Mild	4	Excellent
	7	Male	Sever	5	Excellent
	15	Male	Moderate	5	Excellent
	12	Female	Severe	6	Good
	5	Female	Severe	5	Excellent
	8	Female	Severe	7	Excellent
	10	Female	Mild	5	Excellent
	16	Female	Mild	6	Good
	4	Female	Sever	5	Excellent
Group III patients received f Fucidine cream (market product)	10	Female	Mild	9	Good
	9	male	Sever	13	Faire
	6	male	Sever	10	Faire
	8	female	Mild	9	Good
	5	male	Mild	12	Faire

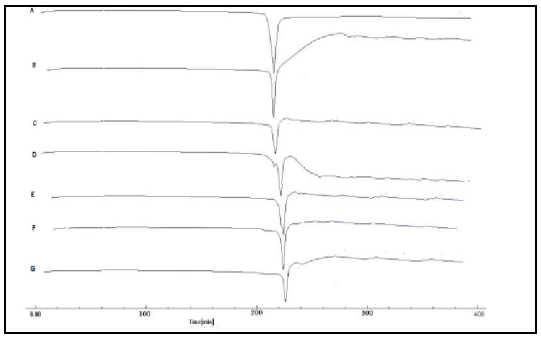


Fig. 1: DSC thermograms of LVFX alone and itsphysical mixtures with the used polymers (1:1w/w); (A) LVFX alone. (B) LVFX: HPMC. (C) LVFX: NaCMC; (D) LVFX: Carbopol934. (E) LVFX: Na alginate; (F) LVFX: P-127. (G) LVFX: PVA 14000.

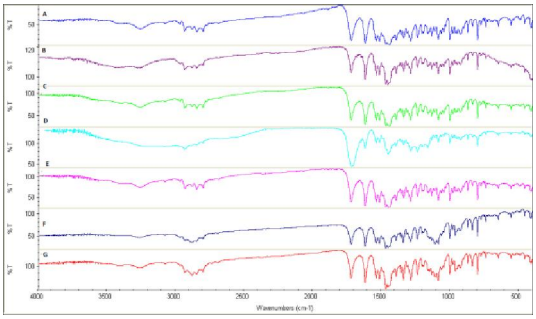


Fig. 2: FT-IR Spectrum of LVFX alone and itsphysical mixtures with the used polymers (1:1w/w); (A) LVFX alone. (B) LVFX: HPMC. (C) LVFX: NaCMC; (D) LVFX: Carbopol934. (E) LVFX: Na alginate; (F) LVFX: P-127. (G) LVFX: PVA 14000.

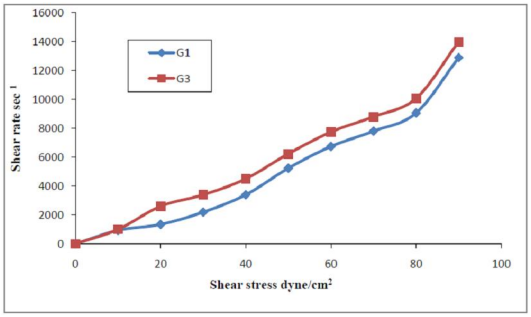


Fig. 3: Rheogram of LVFX - HPMC (2% w/w, G1) and LVFX -NaCMC (3% w/w, G1) gels.

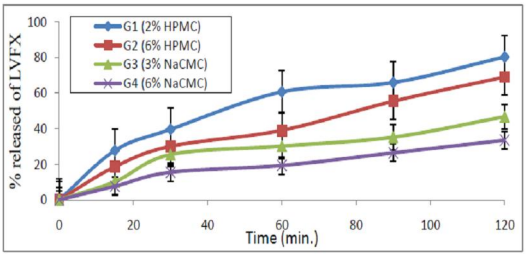


Fig. 4: Release profiles of LVFX from the prepared gels using HPMC (2 & 6% w/w) and Na CMC (3% & 6% w/w) as gelling bases

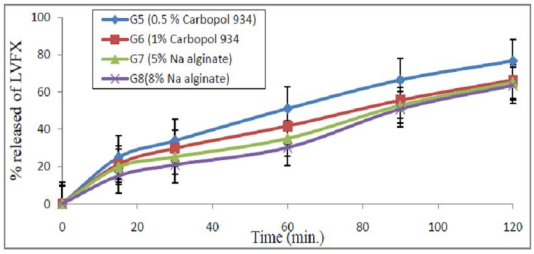


Fig. 5: Release profiles of LVFX from the prepared gels using carbopol 934 (0.5 & 1% w/w) and Na alginate (5 & 8% w/w) as gelling bases

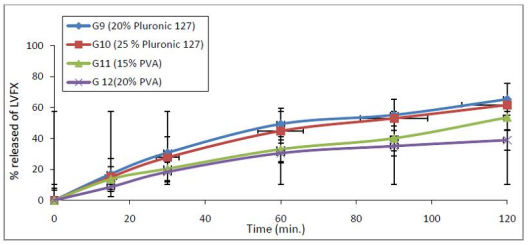


Fig. 6: Release profiles of LVFX from the prepared gels using Pluronic 127 (20 & 25% w/w) and PVA (15 & 20 % w/w) as a gelling bases:

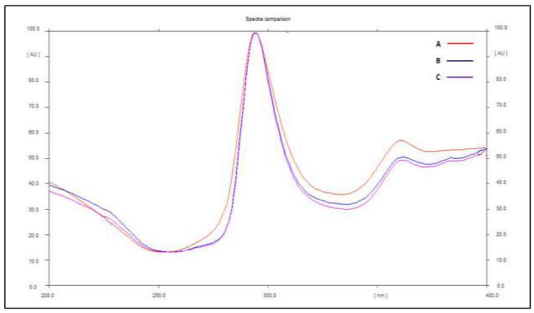


Fig. 7: TLC chromatogram showing (Rf) values of LVFX gel (G1) after 3 months at (A) 5±2^{0}C/ 60±5 %RH, (B) 25±2^{0}C/ 65±5% RH and (C) 40±2^{0}C/ 75±5% RH.

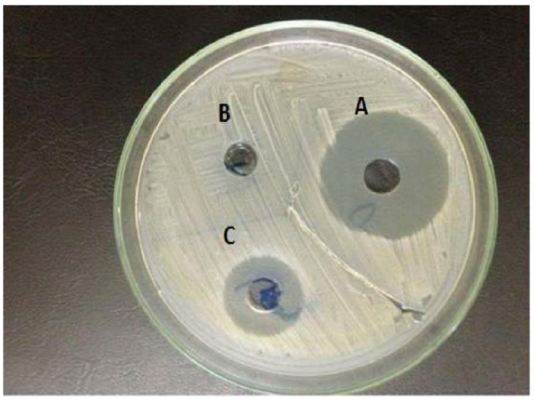


Fig. 8: Inhibition zone of: (**A**): Prepared LVFX gel (G1) against MRSA. (B): Blank gel. (C): Commercial product (Fucidine cream).

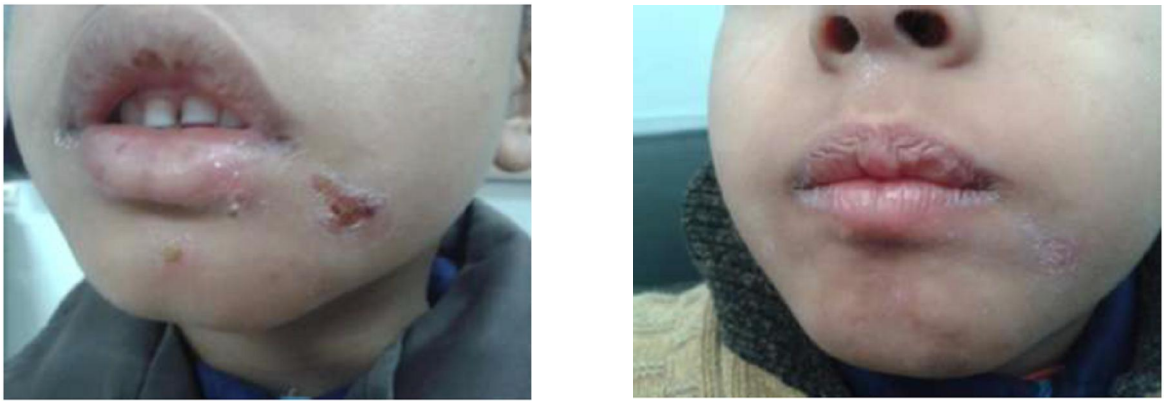


Fig. 9: Left: Ordinary non bullous impetigo in a 7 years boy. Yellowish crusted lesions present around the chin. Right: The same child after only 5 days of treatment with LVFX gel (G1) complete cure and absolute disappearance of impetigo crusts.

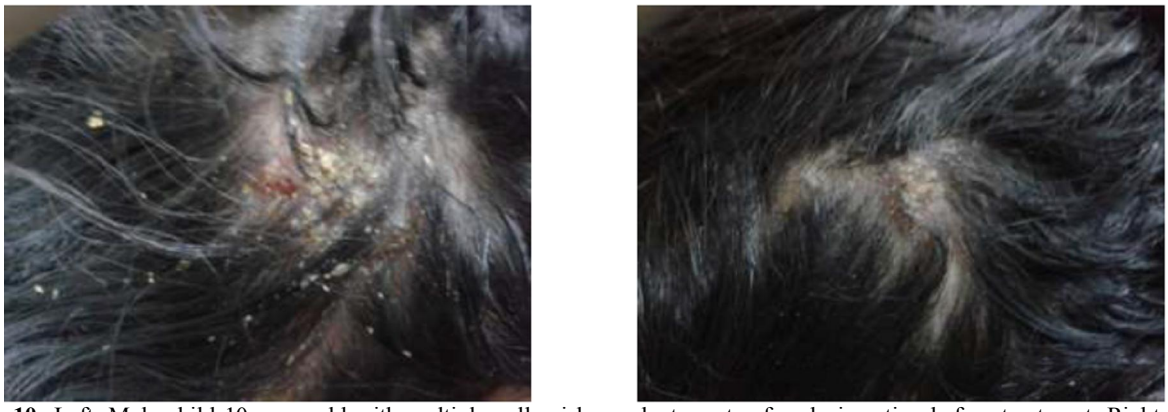


Fig. 10: Left: Male child 10 years old with multiple yellowish purulent crusts of scalp impetigo before treatment. Right: The same patient with good improvement after 4 days treatment with LVFX gel (G1).

Conclusion

The drug is compatible with the investigated gelling agents by using DSC and FT-IR techniques. All the prepared LVFX gels gave acceptable rheological behaviour, in-vitro drug release and have pH suitable for topical applications. The release of the drug from the prepared gels was Higuchi diffusion model and zero order kinetic. Among all the investigated formulae, the best selected one (G1) which gave the highest in vitro drug release and the highest antibacterial activity against MRSA which considered the main cause of impetigo as well as good stability was chosen for further study on clinical cases of impetigo patients. Formula (G1) of LVFX gel showed higher clinical response rates in the reduction of healing time and treatment of impetigo in children under 17 years of age compared with the marketed product (Fucidinecream). Levofloxacin hemihydrate is a potentially useful quinolone for the treatment of skin and soft tissue infections, and its potent bactericidal activity might be able to shorten the treatment period of such infections.

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