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Formulation and Evaluation of Prednisolone Acetate Microemulsion Ocular Gel

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ABSTRACT

Background: Prednisolone acetate is a glucocorticoid used to treat steroid-responsive inflammation repeatedly during the day, and the primary concerns with its 1% suspension and 0.5% commercial ointment include impaired vision and poor patient compliance. **Objective**: The aim of the current study was to create a prednisolone acetate ophthalmic microemulsion-loaded gel to improve dose accuracy, bioavailability, and consequently the efficacy of prednisolone acetate in treating inflammatory ocular disorders and patient adherence. **Material and methods:** A unique formulation was created by combining prednisolone acetate microemulsion with a different gelling agent to create a microemulsion-loaded gel. **Results:** The melting points determined from differential scanning calorimetry (DSC) and the capillary tube technique are extremely close to the standard, indicating the purity of the prednisolone acetate utilized. The best recipe (G2), a microemulsion-loaded gel with 1% carbopol934, had a white milky hue, high homogeneity, spreadability, and gelling capability. The drug content was 99.76%. The pH of the produced gels is comparable to that of eye tears and is not irritating to the eye. The FTIR data show that there is no interaction between the pure medicines and any of the excipients. When integrated into an optimal microemulsion-loaded gel formulation, drug release as obtained in Sorensen phosphate buffer saline (pH 7.4) attained an average of 97.51% in 8 hours. Ex vivo permeation studies further validated the two-fold increase in drug flow through the cornea from MEs-loaded gel compared to marketed ointment.

Conclusion: Prednisolone acetate microemulsion-based gel (G2) ocular drug delivery system provides a potential strategy for enhancing corneal contact, penetration, and flux for ME-loaded gel formulations compared to the control; Extended precorneal retention in the eye resulting in prolonged medication release, increased bioavailability, and patient compliance.

Keywords: Prednisolone acetate, Carbopol934, Microemulsion, In-vitro drug release, Ocular drug delivery.

INTRODUCTION

From an anatomical and physiological perspective, the eye is a special organ since it has a variety of structurally very diverse and physiologically very different parts. For instance, the cornea is a human tissue that is not supplied with blood [(1)]. Protecting internal eye structures, assisting with the eye's refractive power, and concentrating light rays on the retina with the least amount of scatter and optical deterioration are just a few of its crucial corneal tasks [(2)]. The eye, an important visual organ, is composed of two main parts, anterior segment and posterior segment [(3)].

Drug distribution to the eye is a difficult endeavor owing to the eye's intrinsic and complex anatomical and physiological barriers. These barriers are also dependent on the mode of delivery, which might be topical, systemic, or injectable. Because of its convenience of administration and high patient adherence, topical instillation of dosage forms such as solutions, suspensions, and ointments is the recommended method for anterior segment distribution [(4)]. The main issue with these traditional topical administration methods is their limited bioavailability. Furthermore, several precorneal variables impact topical dosage form bioavailability, resulting in less than 5% of the injected dose reaching deeper ocular tissues. Despite this, nanotechnology is now part of a new age in which breakthroughs in new technology have enabled the development of various nanosystems capable of overcoming (the enormous complexity of the eye's structure, including its anatomical and physiological obstacles). The development of materials on a micro scale is characterized as nanotechnology [(5)]. Microemulsions are defined as dispersions of water and oil in the presence of a surfactant and cosurfactant combination (Smix) that reduces interfacial tension. (MEs were categorized as o/w, w/o, or bicontinuous based on the nature of dispersion and disperse phase. These systems are often distinguished by their clear appearance, greater thermodynamic stability, tiny droplet size (<200 nm), and good drug solubility, as well as their use as a drug reservoir for lipophilic and hydrophilic medicines [(6)]. Furthermore, they achieve prolonged release of a medication administered to the cornea as well as deeper ocular structural penetration than the original drug [(7)]. One of the most dreadful aftereffects of intraocular surgery is post-operative endophthalmitis (POE), for which 1% prednisolone acetate (PA) ophthalmic solution must be used at least three to four times daily [(8,9)].

Prednisolone acetate, a glucocorticoid used to treat steroid-responsive inflammation of the palpeberal and

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bulbar conjunctiva, cornea, and anterior parts of the globe, has 3 to 5 times the anti-inflammatory effect of hydrocortisone based on weight. As a Class II medicine in the biopharmaceutics categorization system, prednisolone acetate has poor solubility and high permeability, making it almost insoluble in water. Because of its poor solubility in water, it is sold as a micronized ophthalmic suspension. Due to aggregation, sluggish dissolving rate, and restricted corneal residency, the micro suspension provides poor dosage accuracy and effectiveness [(10)]. Using ophthalmic agents like 0.5% prednisolone acetate ointments has numerous disadvantages. They are generally very sticky causing discomfort to the patient when used. Likewise, they also have low spreadability and necessitate applying with rubbing. They also may show evidence of the problem of stability. Because Prednisolone Acetate dissolves slowly, its bioavailability is also quite poor. By creating a microemulsion and using low-frequency dosage and brief steroid use periods, poor absorption may be enhanced. PA microemulsion gel preparation was chosen as the subject of this research as a result of all these variables, which have boosted the usage of clear gels in pharmaceutical preparations within this category of semisolid preparations [(11,12)].

The aim of the current study was to create a prednisolone acetate ophthalmic microemulsion-loaded gel to improve dose accuracy, bioavailability, consequently the efficacy of prednisolone acetate in treating inflammatory ocular disorders and patient adherence.

MATERIALS AND METHODS

Prednisolone Acetate (PA) purity of 99.4% was purchased from Baoji Guokang Bio- Technology Co., Ltd. Isopropyl myristate, oleic acid, and tween80, were purchased from Taihua Bio. Carbopol 934, carbopol 940, hyaluronic acid, sodium alginate from Zhejiang CP chemical Co, Ltd, China and double deionized water (DDW) were prepared freshly whenever required.

Ethical Approval:

This study was ethically approved by the Institutional Review Board of the University of Baghdad.

Methods

Physical properties determination of prednisolone acetate

Establishing the melting point

The melting point of Prednisolone Acetate (PA) was evaluated using the capillary tube technique outlined in USP. Using a glass capillary tube with one end capped, the PA powder was tapped on a firm surface to form a compact column. The capillary tube is then positioned into the melting point device. The melting point of the medication was determined by progressively increasing the temperature until the powder entirely melted, then stopping the increase and recording the final temperature [(13)].

Differential scanning calorimeter (DSC):

To test PA's thermotropic characteristics, thermal behavior and thermogram, a 3 mg sample was sealed in aluminum pans and heated at 10°C/min from 25 to 300°C using nitrogen as a blank gas in the DSC equipment [(14)].

Determination of λ Max

Prepared and diluted stock solutions of PA (100 µg/ml) in methanol and Sorensen Phosphate Buffered Saline (SPBS) pH 7.40, with 1% Sodium Lauryl Sulphate(SLS) to obtain sink condition were scanned by UV spectrophotometer between 200 and 400 nm to identify the max of PA in each solvent [(10,15,16)].

Determination of Calibration Curves

Calibration Curves of PA in methanol and SPBS pH 7.4 with 1% sodium lauryl sulphate solutions were produced from a stock solution of 100 µg /ml by serial dilutions (10,12,14,16,18,20,22,24,26) µg /ml. Samples were spectrophotometrically analyzed at the wavelength of maximal absorbance of PA. The computed absorbance was recorded and plotted vs concentration to be compared to the relevant concentration. The calibration curve equation and R2 value were obtained.

Diagram of the pseudoternary phase

A pseudoternary phase diagram may be used to assess if a formula will produce a satisfactory microemulsion preparation. The pseudoternary phase diagram depicts the concentrations of water, oil, and surfactants-cosurfactants that will be employed in microemulsion formulations. The water titration technique is used to create a pseudoternary phase diagram. Surfactant and cosurfactant mixtures (Tween 80 and propylene glycol:ethanol (1:1) with ratio 2:1 and oil phase mixtures (oleic acid: isopropyl myristate (IPM) (1:1) were combined in the following ratios: 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. The mixture was progressively shaken to generate a transparent liquid, indicating the formation of a microemulsion as illustrated in **Figure 1**. The pseudoternary phase diagram was created utilizing the findings of the experiments acquired by graphing analysis in Origin 2018 [(17)].

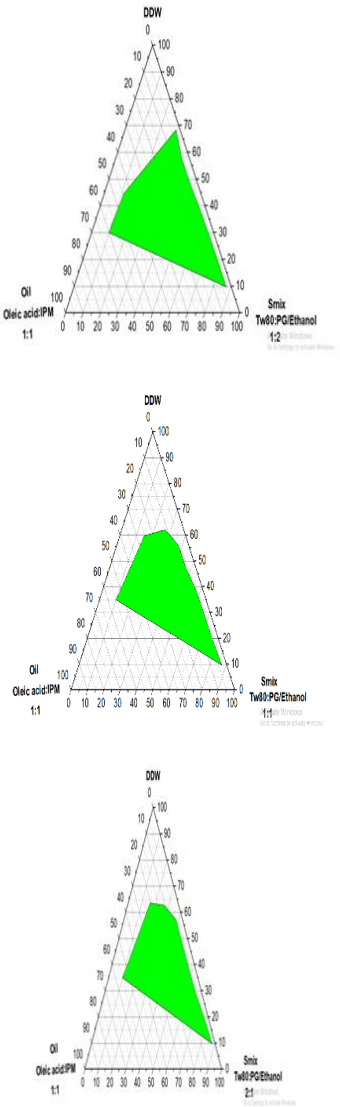

Figure (1): Pseudoternary phase diagram of oleic acid: IPM, Tween80, PG/ ethanol, and SPBS at Smix ratio (1:2, 1:1, 2:1).

Preparation of prednisolone acetate emulgel

Different formulas of prednisolone acetate emulgel were prepared in the table (1). The first step is forming the microemulsion that includes the drug dissolved in the oil phase, using (Oleic acid: IPM) at a ratio (1:1) as the oily phase and the dispersing media for the drug. Tween 80 was used as surfactant and PG: EtOH (1:1) was used as cosurfactant, at a ratio (2:1) of Smix. The benzalkonium chloride (BK) has been frequently employed as a preservative in other commercial eye drops and used in lesser doses as a cationic agent in emulsions has been shown to be safe for the eye, it was dissolved in an aqueous phase as a preservative (18). In the second step forming the gel phase using, carbopol 934, carbopol 940, hyaluronic Acid (HA), and sodium alginate (SA) was used as gelling agent dissolved in water to form the gel, the weight of each formula was adjusted to 100gm.

To achieve 0.5%w/w and 1%w/w concentrations of each gelling agent, 0.5 g and 1 g of each carbopol 934, carbopol 940, hyaluronic acid (HA), and sodium alginate (SA) were distributed in 60 ml of distilled water and stirred with a magnetic stirrer. A few drops of triethanolamine were added to the gelling agent carbopol (934,940) until the pH reached around 6-6.5, the solution thickened, and the gel was produced.

Table (1): Prednisolone acetate ME Based Gel Formulas as (w/w) %

No.	PA	Oil	Smix	Carb 934	Carb 940	HA	SA	BK	Water
G1	0.5	10	30	0.5				0.01	Q. S
G2	0.5	10	30	1				0.01	Q. S
G3	0.5	10	30		0.5			0.01	Q. S
G4	0.5	10	30		1			0.01	Q. S
G5	0.5	10	30			0.5		0.01	Q. S
G6	0.5	10	30			1		0.01	Q. S
G7	0.5	10	30				0.5	0.01	Q. S
G8	0.5	10	30				1	0.01	Q. S

preparation Emulgel preparation

The prepared emulsion (40 gm) of microemulsion was gradually added to 60 gm gel with continuous stirring until uniform (100gm) of emulgel formed.

Evaluation of the Prepared gel

Homogeneity

The formulas were tested for color, homogeneity, consistency, separation, and gelling capacity by visual appearance.

Measurement of pH

Every recipe's pH was determined by placing the pH meter's glass electrode into a screw cap containing emulgel and taking a reading.

Test for ocular irritation

All of the potential PA formulations were put through this test to see whether they would cause any irritation to the eyes (drug ointment and the selected emulgel). The medication ointment was applied to group I (n= 3) and the chosen emulgel was applied to group II (n= 3). The conjunctival sac of the right eye was injected with 50 µl (0.5% w/v PA) of the test formulation, while the left eye served as a control. Inflammation signs such as redness, increased lacrimation, conjunctival edema, and hyperemia were evaluated in both eyes. At 2, 4, 6, 8, 24, and 48 hours, the animals were analyzed [(19)].

Spreadability test

Spreadability is an expression used to determine the extent of the area to which the topical dosage form readily spreads on

application to eye. The therapeutic efficiency of this formulation also depends on its spreadability value.
The diameter of the circle created by the emulgel was measured using two glass plates, 25 g of emulgel, and a second glass plate put on top of the first glass plate. After that, a 500 g weight was put on top of the plates, and the new diameter was recorded [20].

Drug content analysis

The drug content of the formulations was determined by dissolving 200 mg of emulgel corresponding to 1mg of PA in 10 ml of methanol, closing the tube firmly, and placing it in the sonicator for 0.5 hr to thoroughly dissolve the drug. The alcoholic solution was then filtered with a 0.45 millipore filter. The concentration was evaluated in a UV spectrophotometer at 242 nm using methanol as a blank after 1 ml of filtrate was diluted with 10 ml of methanol.

In vitro drug release studies

Six formulas were chosen to investigate their release profile in comparison with the marketed 0.5% PA ointment. Using a modification of Franz cell, type II dissolution apparatus (Campbell electronics, India) was used and 300ml of (SPBS) (pH of 7.40) (21) with 1% of sodium lauryl sulfate (SLS) was used as a dissolution medium at 34±1°C. (0.5 g) from formula(G1,G2,G3 G4, G6, and G8) were placed on dialysis bags (8000-14000D), which were soaked in SPBS overnight prior to the experiment, and the dialysis bags were fitted on the open end of a glass tube in which the emulgel was in the center. The tube was placed in the beaker of dissolution apparatus containing 300ml of SPBS (pH 7.4) with 1%SLS. The apparatus speed was set at 50 rpm and the temperature was maintained at 34±1°C. The study continued for 8 hours, at each time interval (0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8 hours), 5 ml of dissolution medium was withdrawn and replaced with 5 ml of a freshly prepared buffer. The withdrawn samples were analyzed spectrophotometrically at λ max 247nm [22,23].

Ex-vivo permeation investigation

Fresh corneal membrane tissues from a sheep acquired from a nearby slaughterhouse were carefully extracted. It was removed with care and placed in an SPBS until it could be used for the study. Using Franz diffusion cell type b, the corneal membrane was attached between the donor and receiver compartments for the ex-vivo investigation, with the epithelial surface facing the donor compartment holding the test or control formulations.
The G2 was placed in the donor compartment, while 7 ml of SPBS with 1% SLS at a pH of 7.4 was placed in the receptor compartment. At predefined intervals, 1 ml samples were obtained at 50 rpm and 34 degrees Celsius from the receptor chamber. After each sampling, the volume that had been sampled was replaced with new media. The removed samples were analyzed using a UV-VIS spectrophotometer to determine the quantity of medication that had infiltrated.
J = dQ /dt A (µg / cm2 min) ……. . The flux (J)
$\boldsymbol{p} = (\boldsymbol{dQ/dt})/ \boldsymbol{Co \times A}$ ……. The permeability coefficient (p)
$\boldsymbol{ER} = \boldsymbol{flux\ of\ test\ formula}\ /\boldsymbol{flux\ of\ control}$…….. calculate the enhancement ratio (ER).
Permeation parameters were also calculated for PA suspension as a control.

Analysis using the Fourier transform infrared (FTIR)

Prednisolone acetate's IR absorption spectrum and the best emulgel formulation were recorded using the potassium bromide dispersion method, which included placing a drug and potassium bromide combination in a sample holder and recording the infrared spectrum using FTIR (Shimadzu, Japan). The sample's authenticity was established by comparing the discovered peaks to the stated primary peaks of PA's IR spectra [24].

RESULTS

Characterization of Prednisolone Acetate

Prednisolone Acetate (PA) melting point: The PA melting point was 240±1˚C, which matches the reported point and demonstrates the drug powder purity [25].

A differential scanning calorimetry (DSC) study was performed on PA:

As shown in **Figure 2**, the PA pure drug exhibited a characteristic endothermic peak at a temperature of 241.65 °C. This peak indicated that the drug was pure and did not contain any impurities because it was near the melting point, which was detected by electrical melting point instruments, and it was close to the standard reference.

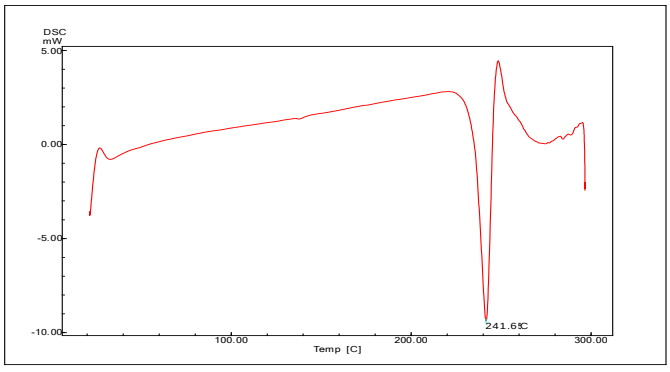

Figure (2): The DSC thermogram of prednisolone acetate.

Maximum wavelength absorbance of PA: PA spectrum that was obtained, the wavelengths of maximum absorbance (λ max of UV scan) of PA in methanol and Sorensen phosphate buffered saline (SPBS) pH (7.4) 1% SLS to reach sink condition [9] (found to be 242 nm and 247 nm, respectively).

Calibration curve of Prednisolone Acetate:
The calibration curve was found to satisfy Beer's law over the concentration range that was investigated, as shown by the production of a straight line with a high regression coefficient, as shown in figure (3) and(4).

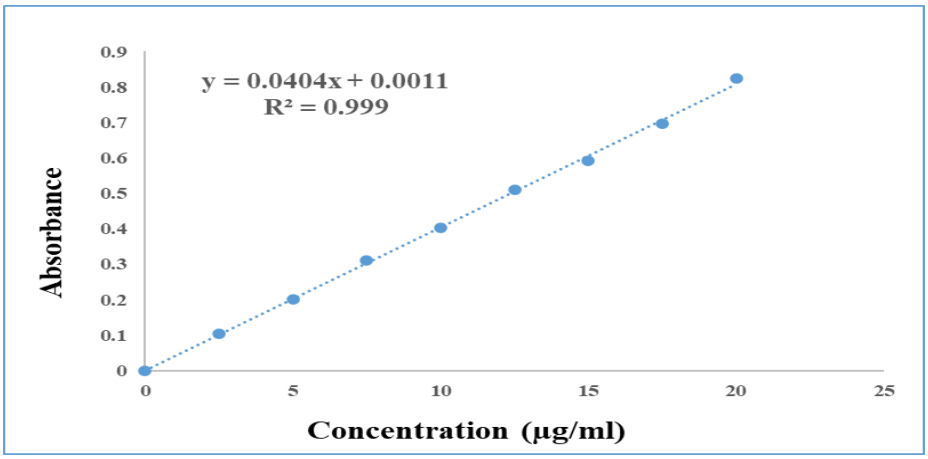

Figure (3): Calibration curve of PA in methanol.

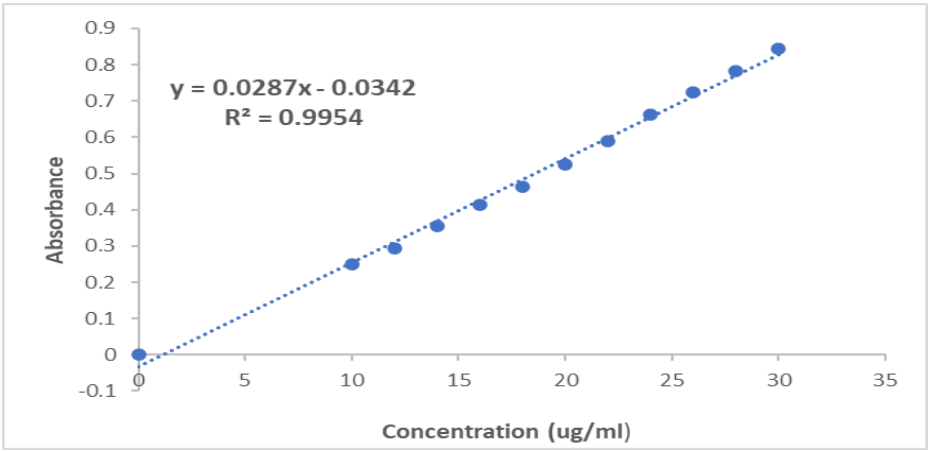

Figure (4): Calibration curve of PA in phosphate buffer pH (7.4) with 1% Sodium Lauryl Sulphate.

Homogeneity: All the prepared formulas were white milky appearance, creamy in consistency except formula (G5 and G7) were very light liquid showing no gelling capacity the results are shown in the table, those formulas failed to give stable emulsion-loaded gel, and were excluded from further studies. No separation was observed before or after mixing of emulsion with the gel as illustrated in **Table 2**.

Table (2): Emulgel Formula Physical Appearance.

No. of formula	Color	Homogeneity	Consistency	Separation	Gelling capacity
G1	White milky	Homogenous	Creamy	No	+++
G2	White milky	Homogenous	Creamy	No	+++
G3	White milky	Homogenous	Creamy	No	+++
G4	White milky	Homogenous	Creamy	No	+++
G5	White milky	Homogenous	Liquid	No	-
G6	White milky	Homogenous	Creamy	No	++
G7	White milky	Homogenous	Liquid	No	-
G8	White milky	Homogenous	Creamy	Yes	++

Note: +: gels rapidly dissolve after minutes, ++: gels instantly remain a gel for hours, +++: gels instantly remain a gel for more than 8 hours, -: no gelation occurred.

pH measurement

The pH values of all created formulations varied from 6.09 to 6.563, as shown in **Table 3**, therefore they were suitable for avoiding discomfort upon application to the eye, since the eye is resistant to changes in pH, i.e., it can tolerate a pH range between 3 and 9 [26].

Table (3): pH Value of Prepared Emulgel Formulas of Prednisolone Acetate

No. of formula	pH	SD	Spreadability	SD	Drug content	SD
G1	6.09	0.18193	3.7033	0.0410	99.06	0.51
G2	6.563	0.3758	3.52	0.0489	99.76	0.05
G3	6.453	0.0960	3.5333	0.0492	99.73	0.15
G4	6.363	0.2557	3.4066	0.0368	99.43	0.20
G6	6.296	0.0513	3.8	0.0326	99.50	0.68
G8	6.45	0.0964	3.6133	0.0464	98.5	0.5

Test for ocular irritation

It was determined that the promising formula G2 caused no irritation on eyes of the test animals, which may be due to the low excipient, surfactant, and cosurfactant concentrations used as well as due to its safety and non-toxicity.

Spreadability test

As the polymer content increases the width of the spreading gel decreases, with values ranging from 3.4066 0.0368 to 3.8 0.0326cm (**Table 3**). For this reason, preparing the gel at a high concentration may restore its high strength and viscosity, since spreadability was shown to be inversely proportional to the degree of the polymer networks' cross-link [27].

Drug content analysis

As indicated in **Table 3**, the drug concentration of the six formulations varied from 99.0666 to 99.7666%, indicating that the medication was evenly dissolved and dispersed throughout the formulations.

In vitro drug release analysis

Figure 5 displays the drug release from each of the six formulations; the formulations containing Carbopol showed the highest release of drug, while the formulations containing hyaluronic acid and sodium alginate showed the slowest release of drug, and it was observed that formulations containing (Carbopol 934) gave better release than formulations which contain (Carbopol 940), since the type or grade of the gelling agent affects the release.

In comparison to the 6 formulas released with the commercially available 0.5% PA ointment, the number of formulas released is larger; this may be attributed to the greater solubility of PA in the microemulsion, the greater hydrophilicity of the emulgel, and the thicker oil found in the ointment's composition [28].

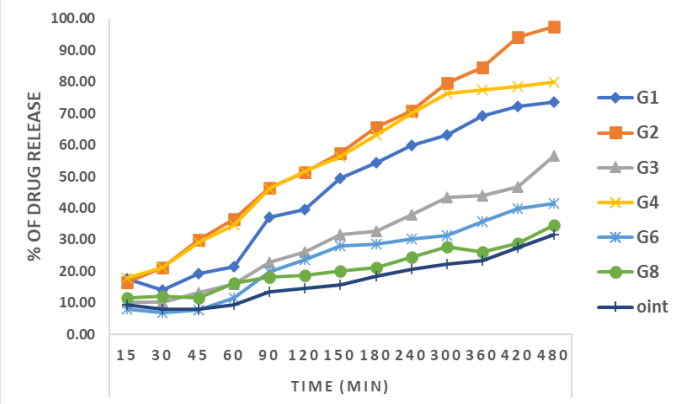

Figure (5): A comparative dissolution profile of prednisolone acetate gel (G1, G2, G3, G4, G6, G8, and prednisolone acetate ointment) in 300ml of SPBS (with 1% SLS) dissolution medium at 34 °C.

Ex vivo study

According to the drug release research, the promising formulae are those that release the medication fast and fully within 8 hours, therefore G2 was exposed to an Ex vivo investigation, the results of which are displayed in **Figure 6**. The best recipe, G2, was the most effective in permeating the medication through the eye cornea. The findings are based on the cumulative quantity penetrated per unit area, steady state flux, permeability coefficient G2, and PA ointment values. Fluxes for G2 and PA ointment were (3.414146 ug/cm2.min) and (1.651162 ug/cm2.min), respectively. In the instance of G2, the permeability coefficient of PA loaded gel raised by 2.0677-fold (0.854 *10-3 cm/s) as compared to the permeability coefficient of PA ointments (0. 413*10-3 cm/s). The difference in the quantity penetrated between the formulations and the control ointment might be related to the fact that obtaining high corneal penetration requires a tiny and uniform droplet size.

Furthermore, oleic acid, Tw80, PG, SLS, and ethanol may improve membrane permeability by functioning as a lipophilic permeation enhancer [29].

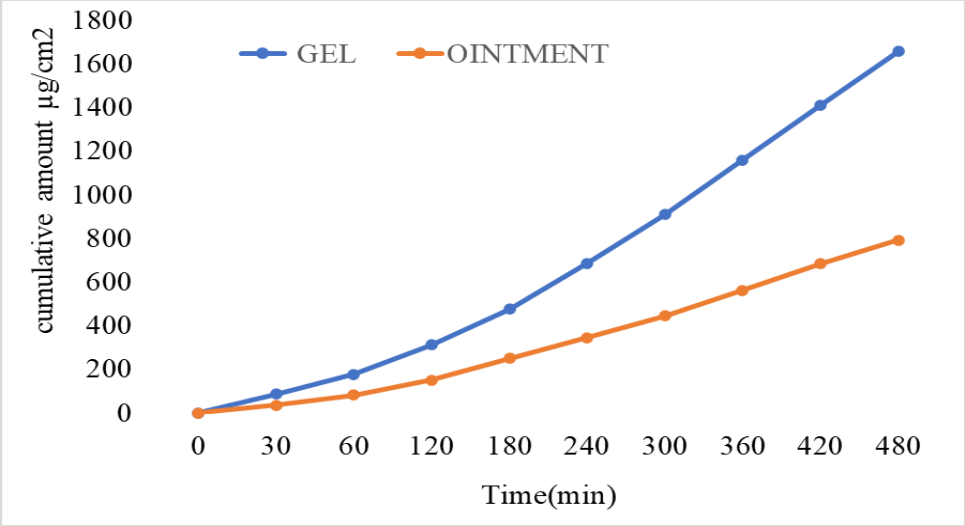

Figure (6): Ex vivo drug diffused from PA emulgel formulas G2 in comparison with 0.5% PA marketed ointment.

Fourier transform infrared spectroscopy (FT-IR)

The FT-IR spectra of PA pure medication, G2, presented in **Figures 7 and 8**, reveal that the best formulae displayed the same functional group's band with some of the stretching frequencies dissipating as a result of PA's solubilization in the formula.

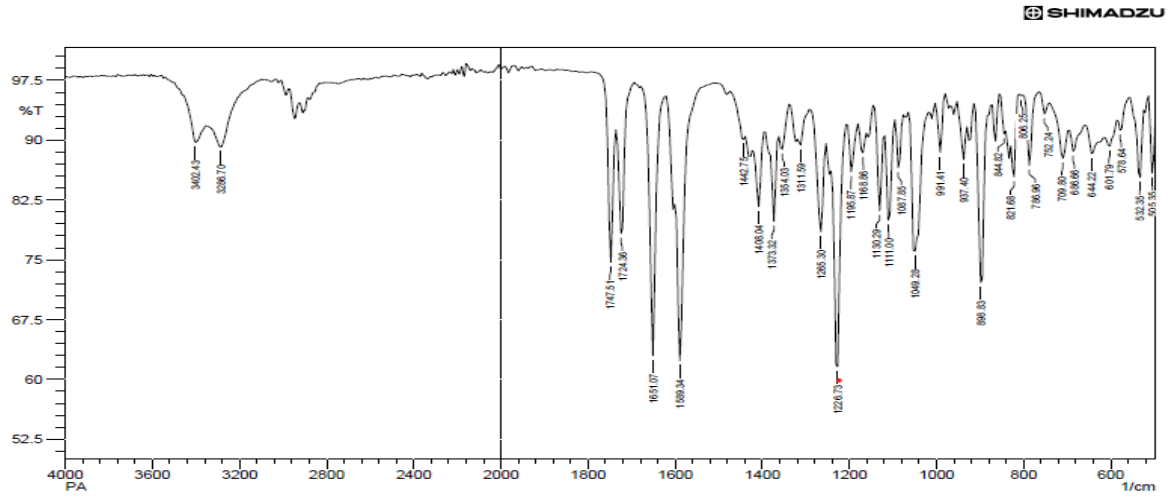

Figure (7): FT-IR Spectrum of Pure Drug PA.

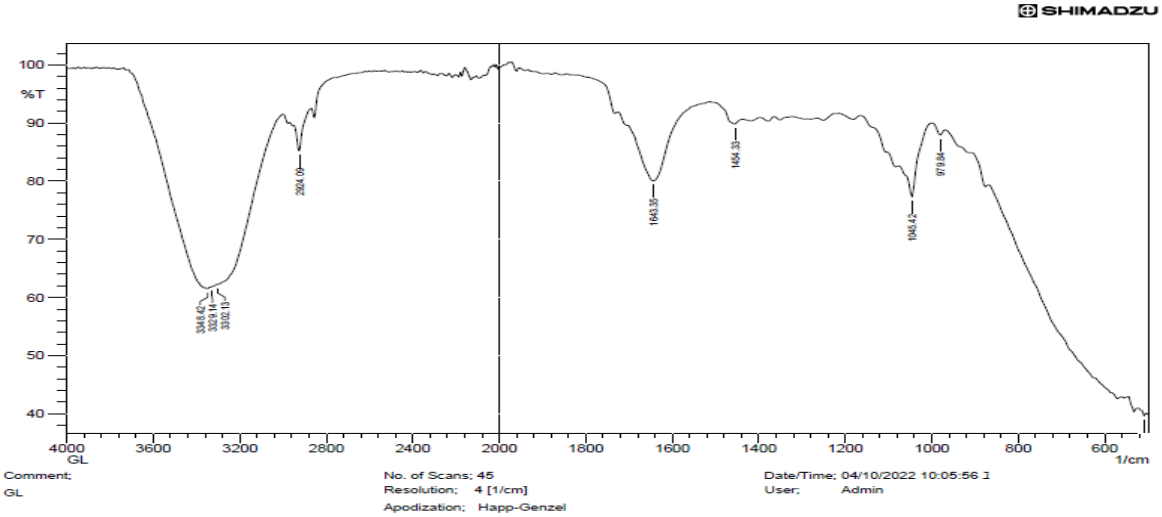

Figure (8): FT-IR Spectrum of microemulsion-based gel (G2).

CONCLUSION

The findings suggest that the prednisolone acetate microemulsion-based gel (G2) ocular drug delivery system provides a potential strategy for enhancing corneal contact, penetration, and flux for ME-loaded gel formulations compared to the control; Extended precorneal retention in the eye resulting in prolonged medication release, increased bioavailability, and patient compliance.

Conflict of interest: The authors declare no conflict of interest.

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