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FORMULATION DEVELOPMENT AND EVALUATION OF SKIN CARE EMULGEL FROM NATURAL OILS

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ABSTRACT

This study was aimed to formulate Linseed oil loaded Emulsion (o/w) based gels, by using various concentrations of rose oil and lemon oil as penetration enhancers for transdermal drug delivery. By using response surface methodology (RSM), different thirteen Linseed oil emulgels were formulated and optimized. All formulations were evaluated for stability studies, physico-chemical characteristics, spreadability, skin protection factor, thermal analysis, FT-IR and antimicrobial activity. *In vitro* drug release study was performed in cellophane membrane through dissolution apparatus (at 37ºC with 100rpm) for 24 hours in release medium at pH 6.8. The results were then statistically analyzed. Among all emulgel formulations, EG6 has shown maximum 96.69% Linseed oil release at higher concentration of Lemon oil with decreased concentration of Rose oil. Analysis of variance (ANOVA) was conducted to evaluate the results exhibited independent variables have remarkable effects ($p < 0.05$) on dependent variables. Multiple linear regression analysis (MLRA) was used to compare the results among different formulations of Linseed oil emulgel. Contour plots and 3D surface plots were also constructed to express the response between independent and dependent variables. All formulations have followed korsemeyer-peppas kinetic model. The optimized formulation having skin protection factor 19 has shown 85% antimicrobial activity, considered suitable for skin care. In summary, combination of penetration enhancers (rose oil and lemon oil) in Linseed oil emulgel can be successfully utilized for transdermal controlled drug delivery. Optimized emulgel has good permeability, prolonged residence time on skin surface and

sufficient SPF, proved its strong anti-microbial activity in case of acne vulgaris and other skin problems.

KEYWORDS: Linseed oil, Emulgel, Response surface methodology, SPF, Antimicrobial activity, Drug release kinetics.

INTRODUCTION

In spite of few drawbacks like poor retention, less spreadability and low bioavailability, Topical drug delivery system is considered favorable and convenient route for delivery of therapeutic ingredients into skin, nose, eye and vagina.[1,2] Recently, interest is developed to use polymers with multiple functions like emulsifiers, gelling agent and thickeners that allow to form stable emulsion by decreasing interfacial tensions and increasing viscosity of aqueous phase and then able to covert emulsion into emulgel (gellified emulsion).[3,4]

Dermatologically, emulgels are considered suitable for skin due to some characteristics as easily spreadable, greaseless, easily removable, non-staining, emollient, pleasing appearance, water soluble and thixotropic.[5] The drug is more solubilized in emulgel form, so more easily penetrate subcutaneously, providing more surface area for pharmacological drug action. Now-a-days in cosmetology, natural oils having anti-aging property and as well as antioxidant action for skin[6] are incorporated in emulgel to overcome the side effects of synthetic drugs. Linseed oil is extremely beneficial for chronic skin conditions, such as rosacea, acne, dermatitis, eczema or psoriasis, the fatty acids act to balance out the skin's own oils and reduce any inflammation, helping the skin to repair. Linseed oil having molecular weight 278.436, is a nutrient-rich triglycerides with a high content of the omega-3 fatty acid, alpha-linolenic acid, insoluble in water, while freely soluble in Methanol and n-Hexane.[7]

The present research work was aimed to develop Linseed oil emulgel that for soothing, shinning, anti-aging, antibacterial, anti-wrinkle and sun protecting action topically.[8,9] Thirteen different batches (EG1, EG2 …EG13) of Linseed oil emulgel were formulated using different ratios of penetration enhancers (Lemon oil and Rose oil) for optimization through Response surface methodology (RSM) at fixed amount of permeation enhancer (propylene glycol) and polymer (Carbopol-940).[10] To investigate drug release kinetics from formulated emulgel, drug release studies were conducted by using cellophane membrane in PBS at pH 6.8 using USP dissolution apparatus (Dissolution Tester).

MATERIALS AND METHODS

Materials

Linseed oil, rose oil, lemon oil, propylene glycol, carbopol-940 polymer, tween 20, span 20, triethanolamine, methanol, n-hexane (Merck German origin) of HPLC grade were purchased from local market. Double Distilled Water was obtained from distillation plant of Department of Pharmaceutics, Bahauddin Zakariya University, Multan.

Evaluation of Linseed oil in Phosphate buffer solution (at pH 6.8)

10mg of Linseed oil was mixed in 100ml phosphate buffer solution (pH 6.8) aimed to make stock solution. Different dilutions were prepared from it having concentration range 1-5 µg/mL and then analyzed through UV spectrophotometer (PerkinElmer, lambda 25) at 290nm. Calibration curve was drawn having regression equation Y= 0.0219x + 0.1325 with R^2 0.999.

Determination of Linseed oil Solubility and Partition coefficient (Ko/w)

The solubility of Linseed oil was determined in methanol, n-hexane and phosphate buffer saline (at pH 6.8). An excessive amount of Linseed oil was added in each solvent (5ml) under controlled thermostatically stirring at 37℃ for 72 hours (until thermodynamic equilibrium obtained). Each equilibrated samples were separated and kept for centrifugation at 13000 RPM for 10-15 minutes. The supernatant aliquot was separated and filtered through 0.2µm nylon filter (Fisher scientific, UK). The appropriate dilutions were made in each relevant solvents and analyzed through UV Spectrophotometer at 290nm. The solubility of Linseed oil in each solvent was determined in triplicate at room temperature (25 ± 0.5°C) and results were calculated as mean ± SD.

A small amount of Linseed oil was added in 5 ml distilled water in separating funnel and shaken for 10 minutes. Then 5 ml octanol was added in this mixture and shaken for further 5 minutes and allowed to keep it for 24 hours. The two layers were collected separately and centrifuged at 13000 rpm for 5 minutes. Each layer was analyzed at 290 nm through UV Spectrophotometer after making appropriate dilutions. The solubility of Linseed oil was determined in both solvent and calculate its partition coefficient in octanol/water in triplicate manner at room temperature (25 ± 0.5 °C).

Preparation of Linseed oil emulgels

Linseed oil emulgels were prepared by using varying concentration of penetration enhancers (lemon oil and rose oil) and surfactants (Tween 20 and Span 20) according to RSM as shown in table 1.

For formulating Linseed oil emulgel, weight of each ingredient was adjusted according to their respected HLB (Hydrophillic-Lypophyllic Balance ratio).[11-13] The gel phase was prepared by mixing the required weight of carbopol-940 in sufficient quantity of distilled water on continuous stirring to make lumps free. The oil phase of emulsion was formed by mixing required amount of span 20 in liquid paraffin (co-surfactant). Then added rose oil and lemon oil (penetration enhancers) one by one in oil phase on continuous stirring. Linseed oil was dissolved in methanol in separate volumetric flask and then mixed with oil phase on continuous stirring. Aqueous phase of emulsion was prepared by mixing tween 20 in small amount of distilled water. In required amount of propylene glycol (permeation enhancer), chloroform in water (0.055%) was added as preservative. Mixed this solution with aqueous phase on continuous stirring. Both oil and aqueous phases were heated separately at 70-80ºC for 5-8 minutes and then cool them at room temperature. After cooling, oil phase was added slowly in aqueous phase on continuous stirring to make O/W emulsion (having HLB value 8.7-8.8). It is necessary to add these phases at room temperature because at high temperature droplets of oil will coalesce and at very low temperature, they will freeze. Mixed this O/W emulsion into gel phase on continuous stirring by aid of magnetic stirrer. pH was adjusted at 6.8 by adding Triethanolamine drop wise and required weight was obtained by adding distilled water on continuous stirring until required consistency was attained. These emulgel formulations were stored in aluminum collapsible tubes for evaluation.[14]

Experimental design

Response surface methodology for optimization of Linseed oil emulgel formulations has utilized Design Expert [trial version 7.3, State-Ease Inc. Minneopolis, MN, and USA] by using CCD (central composite design) considering (α=2) as per standard protocol. The amounts of lemon oil and rose oil were selected as factors and studied at five levels each. The central point (0, 0) was studied in quintuplicate.[15-17] The all other formulation variables were kept invariant throughout the study.

Physical evaluation of Linseed oil emulgel

Physical evaluation of all Linseed oil emulgel formulations including homogeneity, transparency, viscosity, texture, drug content and pH were inspected.[18]

pH determination

pH of each formulation was measured by pH meter (Mettler & Toledo, Giessen, Germany) at room temperature (25 ± 0.5 °C).

Rheological study

(Brookfield engineering labs, model rvtdv 11, inc, Stoughton) having 63 spindle at room temperature (25 ± 0.5 °C).

Visual appearance

Each Linseed oil formulation was analysed by naked eye for its uniformity, colour, texture, phase separation and for any aggregates/lumps.

% drug yield and drug content uniformity

The % drug yield of each formulation was calculated by measuring the theoretical mass at the time of preparation and practical mass after 24 hours by using following formula[19];

$$\%\,\text{yield} = \frac{\text{Practical mass}}{\text{Theoratical mass}} \times 100 \qquad (1)$$

The drug content in each Linseed oil emulgel formulation was determined by adding 1gm of each formulation in 100 ml emulgel phosphate buffer solution (at pH 6.8) and shake it well. After filtration, appropriate dilution was made and analyzed through UV Spectrophotometer at 290nm at room temperature (25±0.5°C). Repeat the same procedure with blank sample (without drug) and determined the drug content by following formula;

$$\text{Drug contet} = \text{Absorbance of blank} / \text{Absobance of sample} \times 100 \quad (2)$$

Spreadability

The spreadability of each emulgel formulation was determined by placing the approximately1gm formulation, pointing 2cm circle between two glass slides (15 x 10.5 cm and 5mm) slides[20] while placing the 5gm weight on the upper glass slide for 5 minute.[21]

After this, measured the diameter (in cm) of spreaded emulgel pointed circular area[14] and calculate the spreadability by using following formula;

$$S = \frac{M \times L}{T} \qquad (3)$$

Whereas; S is for spreadability, M is for mass of weight (gm), L is for length of spreaded emulgel circular area (cm) and T is time (sec) taken to separate completely both glass slides. The same procedure is repeated in triplicated manner at room temperature (25 ±0.5℃).

Extrudibility test

Extrudibility measured the flow ability of emulgel formulation from aluminum collapsible tube. For this test, 20gm of each formulation was filled in aluminum collapsible tubes and placed 1kg weight on the flat surface at closed end of tube for 25-30 seconds. The quantity of extruded emulgel from each tube was noted and calculated extrudibility (extrusion pressure/gram).[22]

Evaluation of Linseed oil emulgel

All the prepared emulgels were subjected to evaluation for following parameters;

Fourier transforms infrared spectroscopy (FT-IR)

FT-IR spectra of linseed oil, rose oil, lemon oil, polymer and formulated emulgels were recorded to confirm structural parameters of formulations by using FT-IR Spectrophotometer (Perkin Elmer-spectrum RX-I, Lambda USA). It is analytical tool which is used for observing the interaction between drug and polymer by using KBr Disc method to make pellets by applying 600kg/cm^2 hydraulic pressure. FT-IR spectrum was scanned and values were recorded over range 3500-1000cm^{-1} wave number.

Thermal analysis

TGA was performed on optimized Linseed oil emulgel. 190mg sample was placed in aluminum pan, switching to nitrogen gas at 20.0 ml/min, kept for 1.0 minute at 40°C and then heated from 40-400°C in nitrogen atmosphere at 10°C/ min by PerkinElmer thermal analysis. The thermogram peak was recorded.

Drug release study

In vitro drug release study of all formulated emulgels was carried out in dissolution apparatus (USP type-II) by placing the 1gm each emulgel formulation in cellophane membrane, tied firmly with paddle by thread, using 500ml release medium of PBS (pH 6.8) at 37±2°C on constant stirring with 100 RPM.[23] After appropriate time intervals, sample was taken out, replaced by fresh sample in dissolution apparatus, and then analyzed for absorbance at 290nm through UV spectrophotometer. The concentration of linseed oil in each emulgel formulation was calculated by using regression equation and repeated in triplicate for accuracy.

Drug release kinetics

The fate of linseed oil release was determined by fitting drug release data to five kinetic models (zero order, first order, higuchi, Hixson crowell, korsemeyer-peppas model) through DD Solver (version 1.0)[24,25] For validity of best fit model among all of these mentioned models, AIC (akaike information criterion) was applied through use of DD Solver.[26]

Statistical analysis

Microsoft Excel, version 2013, was used to carry out statistical data analysis including calculation of mean and standard deviation. Statistically significant differences among various parameters of 13 different formulations were determined by using the regression analysis and analysis of variance (ANOVA) with $p < 0.05$ as a minimal level of significance.[27]

RSM Optimization data

In this present research, computerized based streamlining method with RSM usage of polynomial equation has been utilized.[28] Polynomial equations with the interaction and quadratic terms were formulated for response **Y** (% drug release in PBS at pH 6.8) by use of Multiple Linear Regression Analysis (MLRA) approach. The total % amount of Linseed oil was drawn as a function of time. Contour plots and 3D images were plotted to select the formulation variables required to generate desired value.

Determination of Sun Protection Factor (SPF)

1gm of each emulgel sample was added in 100 ml ethanol and then allowed it for ultra-sonication for 10 minutes. After filtration, 5ml of aliquot was transferred in 50ml volumetric flask and diluted it with ethanol up to mark. Again filtered it and 5ml of aliquot was transferred to 25ml volumetric flask and diluted it with ethanol up to mark. To determine the

sun protection factor, a mathematical equation was developed which put *in vitro* method using UV spectrophotometry[29,30] by measuring absorbance at 290-320 ± 5nm, against ethanol as reference.

$$SPF_{spectrophotometric} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda) \quad (4)$$

Whereas; EE (Erythemal effect spectrum), CF[Correction factor (=10)], Abs (Absorbance of sunscreen product), I (Solar intensity spectrum), EE*I values are constant for 290-320nm range from 0.0150 to 0.0180.[30]

Antibacterial activity

The all formulated emulgels were evaluated for their antibacterial activity through Ditch plate technique. Nutrient agar media was used for bacterial growth to see the bactericidal/bacteriostatic activity (Staphylococcus epidermidis). Then took the fresh pus from face pimple of any volunteer via the cotton and applied this pus by sterilized loop onto agar plate. Observed the bacterial growth at these plates after 24 h at 25ºC±0.5. Then added optimized emulgel (1gm) in this agar plate. Now streak across the agar at right angle to the edge of plate and incubate for 24 h at 25ºC±0.5. By using crystal violet dye, checked microbial growth under microscope. Length of inhibition was measured and then calculated the % Inhibition.[31]

$$\%Inhibition = \frac{\text{Total length of inhibition}(L_2)}{\text{Total length of streaked culture}(L_1)} \times 100 \quad (5)$$

Skin irritation test

About 1gm of optimized Linseed oil emulgel was applied on the small area of skin of fore arm of 10 human volunteers for 7 days. They were kept under observation for appearance of any rash, abrasion, itching, burning or allergy.

Accelerated stability test

All formulations were kept under stability chamber for period of six months at accelerated temperature (40±0.5^{o}C) and relative humidity (75±1%). Physical characteristics, pH value and % drug content, rheology and spreadability were examined after every one week, two weeks, one month, three months and six months by similar method previously mentioned.

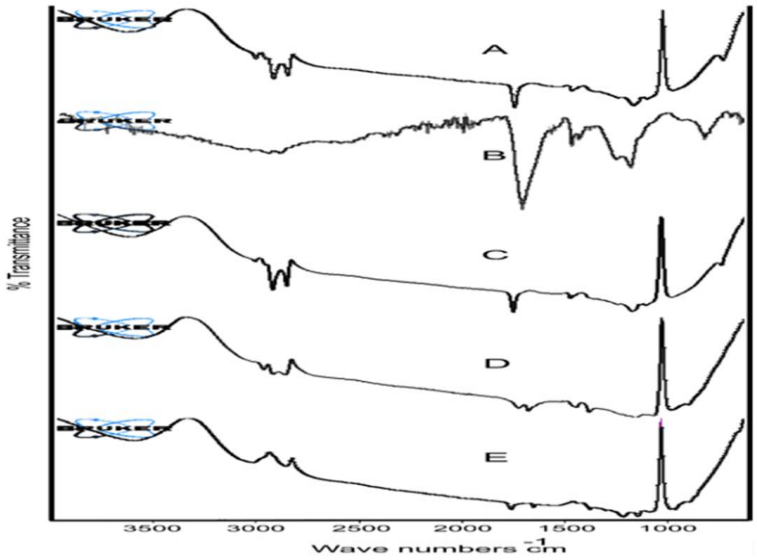

Figure 1:

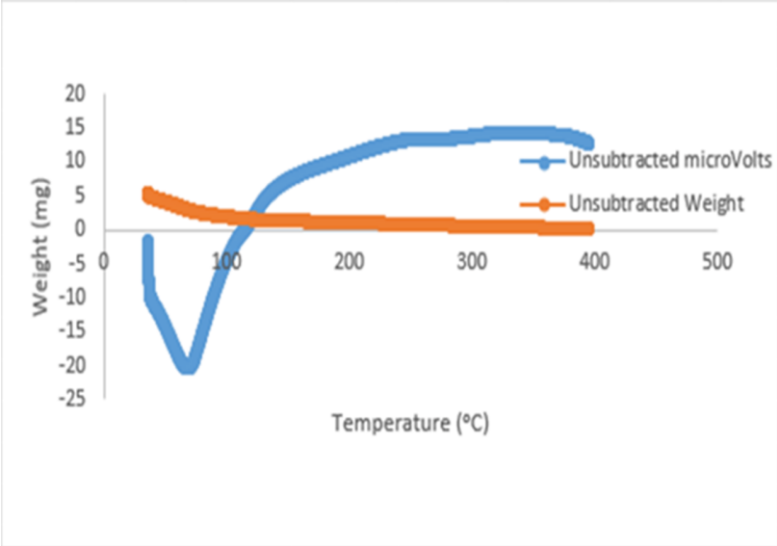

Figure 2:

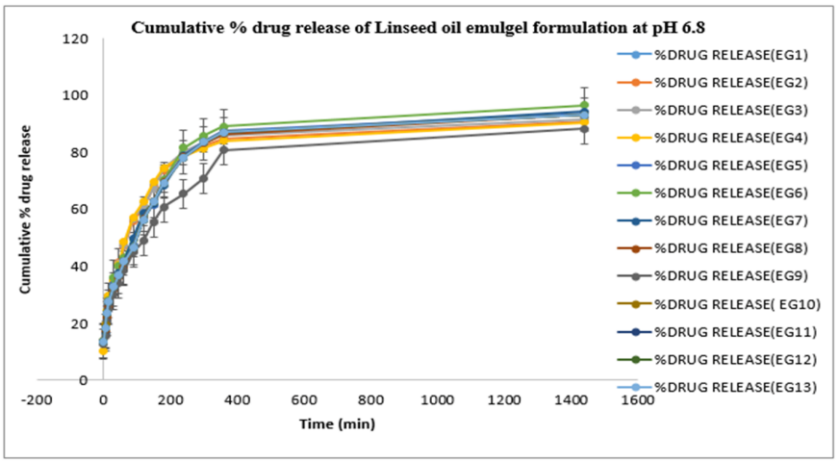

Figure 3:

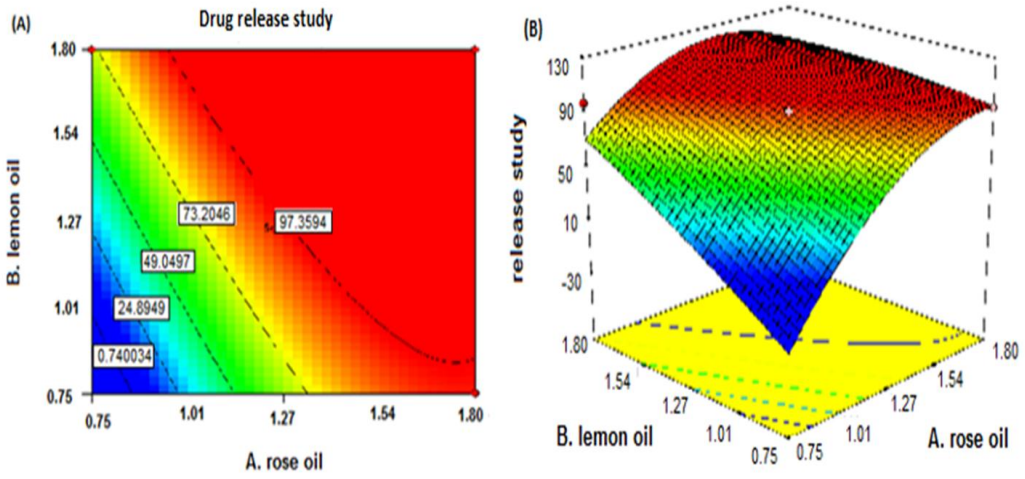

Figure 4:

Table 1: (a)

Trial#	Coded Factor levels		Rose oil (g)	Lemon oil(g)
	$X_{1\ (Rose\ oil)}$	$X_{2\ (Lemon\ oil)}$		
EG1	0	0	1.25	1.25
EG2	2	2	2	2
EG3	1	1	1.8	1.8
EG4	1	2	1.8	2
EG5	1	-1	1.8	0.75
EG6	-1	1	0.75	1.8
EG7	2	1	2	1.8
EG8	-2	1	0.5	1.8
EG9	1	-2	1.8	0.5
EG10	0	0	1.25	1.25
EG11	0	0	1.25	1.25
EG12	0	0	1.25	1.25
EG13	0	0	1.25	1.25

Table 2:

Code level	**-2**	**-1**	**0**	**1**	**2**
$X_{1\ (Rose\ oil)}$ (gm)	0.5	0.75	1.25	1.8	2
$X_{2\ (Lemon\ oil)}$(gm)	0.5	0.75	1.25	1.8	2

Table 3:

Time (min)	EG1 (%)	EG2 (%)	EG3 (%)	EG4 (%)	EG5 (%)	EG6 (%)	EG7 (%)	EG8 (%)	EG9 (%)	EG10 (%)	EG11 (%)	EG12 (%)	EG13 (%)
0	13.58 ±0.01	13.35 ±0.01	14.04 ±0.01	10.4± 0.1	12.90 ±0.01	14.04 ±0.01	13.36 ±0.01	13.58 ±0.01	12.67 ±0.01	13.58 ±0.01	13.58 ±0.01	13.58 ±0.01	13.58 ±0.01
5	19.75 ±0.01	18.61 ±0.01	19.06 ±0.01	19.75 ±0.01	17.24 ±0.01	17.24 ±0.01	16.78 ±0.01	17.47 ±0.01	15.64 ±0.01	19.7± 0.01	19.7± 0.01	19.7± 0.01	19.7± 0.01
10	23.4± 0.01	24.1± 0.1	23.86 ±0.01	24.54 ±0.01	21.35 ±0.01	22.72 ±0.01	22.03 ±0.01	21.80 ±0.01	20.66 ±0.01	23.4± 0.01	23.4± 0.01	23.4± 0.01	23.4± 0.01
15	27.73 ±0.01	28.88 ±0.01	29.57 ±0.01	29.56 ±0.01	25.00 ±0.01	28.20 ±0.01	26.60 ±0.01	25.68 ±0.01	24.77 ±0.01	27.73 ±0.01	27.73 ±0.01	27.73 ±0.01	27.73 ±0.01
30	32.99 ±0.01	34.8± 0.1	35.27 ±0.01	35.04 ±0.01	33.68 ±0.01	36.19 ±0.01	32.31 ±0.01	31.85 ±0.01	30.02 ±0.01	32.99 ±0.01	32.99 ±0.01	32.99 ±0.01	32.99 ±0.01
45	37.10 ±0.01	41.44 ±0.01	40.98 ±0.01	40.75 ±0.01	36.64 ±0.01	40.07 ±0.01	37.79 ±0.01	36.19 ±0.01	34.13 ±0.01	11.39 ±0.01	11.39 ±0.01	11.39 ±0.01	11.39 ±0.01
60	41.66 ±0.01	46.9± 0.1	47.37 ±0.01	48.51 ±0.01	40.75 ±0.01	43.04 ±0.01	41.89 ±0.01	39.61 ±0.01	38.47 ±0.01	11.57 ±0.01	11.57 ±0.01	11.57 ±0.01	11.57 ±0.01
90	46.46 ±0.01	56.1± 0.1	56.74 ±0.01	57.19 ±0.01	47.60 ±0.01	49.43 ±0.01	49.89 ±0.01	46.23 ±0.01	44.86 ±0.01	12.53 ±0.01	12.53 ±0.01	12.53 ±0.01	12.53 ±0.01
120	56.50 ±0.01	60.01 ±0.01	61.07 ±0.01	62.67 ±0.01	56.96 ±0.01	58.33 ±0.01	58.56 ±0.01	56.74 ±0.01	48.97 ±0.01	12.57 ±0.01	12.57 ±0.01	12.57 ±0.01	12.57 ±0.01
150	62.67 ±0.01	67.69 ±0.01	67.24 ±0.01	69.52 ±0.01	61.76 ±0.01	62.44 ±0.01	61.99 ±0.01	62.90 ±0.01	55.59 ±0.01	13.58 ±0.01	13.58 ±0.01	13.58 ±0.01	13.58 ±0.01
180	69.29 ±0.01	73.85 ±0.01	74.32 ±0.01	74.54 ±0.01	69.75 ±0.01	70.43 ±0.01	68.38 ±0.01	69.75 ±0.01	60.84 ±0.01	14.26 ±0.01	14.26 ±0.01	14.26 ±0.01	14.26 ±0.01
240	78.19 ±0.01	79.3± 0.1	80.25 ±0.01	78.88 ±0.01	79.11 ±0.01	81.62 ±0.01	79.11 ±0.01	78.42 ±0.01	65.41 ±0.01	78.19 ±0.01	78.19 ±0.01	78.19 ±0.01	78.19 ±0.01
300	83.67 ±0.01	82.3± 0.1	83.45 ±0.01	81.4± 0.1	83.90 ±0.01	85.96 ±0.01	83.22 ±0.01	83.22 ±0.01	70.89 ±0.01	83.67 ±0.01	83.67 ±0.01	83.67 ±0.01	83.67 ±0.01
360	87.10 ±0.01	84.8± 0.1	86.19 ±0.01	84.13 ±0.01	87.56 ±0.01	89.16 ±0.01	86.87 ±0.01	86.42 ±0.01	80.94 ±0.01	87.10 ±0.01	87.10 ±0.01	87.10 ±0.01	87.10 ±0.01
1440	93.26 ±0.01	90.98 ±0.01	91.67 ±0.01	90.52 ±0.01	93.72 ±0.01	96.69 ±0.01	94.41 ±0.01	93.26 ±0.01	88.24 ±0.01	93.26 ±0.01	93.26 ±0.01	93.26 ±0.01	93.26 ±0.01

Parameter	EG1	EG2	EG3	EG4	EG5	EG6	EG7	EG8	EG9	EG10	EG111	EG12	EG13
kKP	15.42	18.03	18.19	18.73	14.92	15.85	15.20	14.77	13.18	15.42	15.42	15.42	15.42
N	0.269	0.246	0.246	0.240	0.275	0.270	0.273	0.276	0.278	0.269	0.269	0.269	0.269
R^2	0.888	0.886	0.883	0.895	0.887	0.894	0.895	0.884	0.917	0.888	0.887	0.889	0.886
AIC	108.9	108.5	109.3	107.5	109.8	109.1	108.5	110.0	101.2	108.9	108.7	108.6	108.9

Table 4:

Source	Sum of Squares	df	Mean Square	F-Value	P-Value Prob>F	
Model	4913.44	5	982.69	4.40	0.0394	Significant
A-rose oil	721.87	1	721.87	3.23	0.1154	Non-significant
B-lemon oil	342.00	1	342.00	1.53	0.2560	Non-significant
AB	240.26	1	240.26	1.07	0.3344	Non-significant
A^2	1591.28	1	1591.28	7.12	0.0321	significant
B^2	28.04	1	28.04	0.13	0.7337	Non-significant
Residual	1565.02	7	223.57	-	-	-
Lack of fit	1565.02	3	521.67	-	-	-
Pure error	0.000	4	0.000	-	-	-

RESULTS AND DISCUSSION

Determination of Linseed oil Solubility and partition coefficient (Ko/w)

The solubility of linseed oil in n-hexane was 0.00407±0.25mg/ml, 0.00185±0.45mg/ml in methanol, 0.00084±0.67 mg/ml in PBS (at pH 6.8).

The Partition coefficient (Ko/w) for linseed oil was 3.6. From this value, it was shown that given drug comprised of about sufficient lipophilicity that is beneficial to develop the topical drug.[32]

Physical evaluation of Linseed oil emulgel formulations

Physical characteristics like homogeneity, texture, pH, phase separation, viscosity and smoothness of all prepared linseed oil emulgels were observed.

Results has shown that all linseed oil emulgel formulations were smooth, good homogeneity, transparent and lumps free. pH value of all formulations was lied in range of 6.7-6.8 ± 0.1, considered suitable for skin application. All formulations have good consistency as the viscosity of these has lied in range of 640-671*10^3 (cps).

Spreadability values of all linseed oil emulgel formulations were in range of 0.034±0.1 to 0.046± 0.1 g.cm/s while extrudibility values of these formulations were lied in range of 0.95± 0.01 to 1.31 ± 0.01g/cm. Both these parameters have indicated that EG6 easily spread (0.042±0.1 g/cm) on applying small amount of the shear stress and having good extrudibility value (1.27± 0.01) proving its excellent consistency as compared to others.

The changes were observed on human volunteers for any skin irritation/lesion/abrasion after each day and has reported no any lesion, skin irritation or abrasion on skin, confirmed its suitability to skin.

The physical evaluation has revealed good homogeneity, transparency, viscosity, extrudibility, spreadability and stability for prolonged time period. The optimized linseed oil emulgel has no skin irritation and has shown excellent results for skin care. The provided results are in accordance with previous reports.[14,33]

Determination of % drug yield and drug content uniformity test

This study was done to examine the percentage drug yield of all prepared linseed oil emulgels for six months' time period, indicating that % drug yield was in range of 95.5±0.2 to 99.6±0.1 while the drug content of all formulations were in range of 94.8±0.1 to 99.6±0.1. The drug contents of all emulgel formulations were increased by increasing the concentration of lemon oil and at decreased concentration of rose oil. The similar findings have been reported in previous studies.[34]

Evaluation of Linseed oil emulgel formulations

Fourier Transform Infrared spectroscopy (FT- IR)

The FT-IR spectra in Figure 1 has shown no significant difference in polymer (carbopol-940), pure linseed oil, rose oil, lemon oil and optimized linseed oil emulgel (EG6). The peaks in range of 3000-3500/cm was due to alkane group (-CH3) and these were sharper in all spectrum except polymer because of the coordination of linkages. Some peaks were appeared in range of 1600 -2395/cm were due to the alkene group(C=C) and this was sharper in polymer spectra as compare to others spectrum. This has been indicating strong bond interaction among alkene group of polymer. Whereas, peaks in range of 1020-1160/cm were due presence of phenyl group. Results of FTIR spectra of linseed oil were found to be in good agreement and suggested the linseed oil stability in emulgel formulation with respect to carbopol-940 and penetration enhancers (rose oil and lemon oil).

Thermal analysis

The stability of linseed oil in Carbopol-940 was investigated by thermal analysis using TGA thermograms. The melting point of drug loaded optimized EG6 emulgel of linseed oil was revealed by the exothermic single sharp peak at -23ºC. The loading temperature was 30ºC.

The result of thermal analysis proved the stability of linseed oil emulgel at molecular level. The TGA curve of optimized emulgel EG6 is shown in figure 2.

In vitro drug release study

The release of Linseed oil from all emulgel formulations was analyzed for 24 h and calculated the release amount by using regression equation for calibration curve y = 0.0219 x + 0.1325 with regression coefficient R^2 = 0.9994 at pH 6.8. The results indicated that formulated linseed oil emulgel EG6 has shown the highest drug release (96.69%±0.01). Cumulative % drug release profile of all linseed oil emulgel formulations at pH 6.8 (n=3±SD) has shown in Table2 and Figure 4. The drug release profile of all emulgel formulations (EG1, EG2,..,EG13) at pH 6.8 showed abrupt release of linseed oil from all formulation due to high concentration of rose oil except EG6 that showed its maximum release in moderate manner in lesser time having increased concentration of lemon oil and decreased concentration of rose oil. These findings are in accordance with the pervious published reports.[33,35]

Drug release kinetics

The mode of drug release of has followed Korsemeyer-peppas model, considered as most suitable model for all formulated Linseed oil emulgels at 6.8 pH due to the greatest coefficient of determination value (R^2) and lowest AIC value among other models as shown in Table 3, indicating that mode of drug release was not dependent on concentration of drug. The emulgel formulation has shown Fickian diffusion as n<0.45.

RSM Optimization data modeling

The multiple linear regression analysis was utilized for creating a relationship mathematically and expressed as polynomial equation. The positive value of coefficient depicts synergistically effect while negative value shows antagonistically effect on response. The higher value of coefficient indicates that the factor has the strong impact upon response. The result of Multiple Linear Regression Analysis of response has shown % Co-efficient of variation (17.33%), F-value (3.72), R^2 (0.75) and mean ± SD (86.29 ± 14.95).

Effect of enhancers on % drug release at Y (pH 6.8)

Significance probability P value (p>0.05) for response Y depicts that linear participation Rose oil (A) and Lemon oil (B) has produced non-significant effect (p< 0.05) synergistically. On the other hand, the cross product participation of Rose oil and Lemon oil (AB) also produced non-significant effect (p<0.05) antagonistically while quadratic contribution A^2

produce significant ($p>0.05$) antagonistic effect while B^2 produce non-significant ($p<0.05$) effect antagonistically. This has been shown in ANOVA table 4.

The polynomial equation is given here in terms of coded factors as:

$Y= 96.75+38.28A +26.35B -20.79AB – 30.68 A^2-4.07 B^2$

The above equation reveals that Rose oil (A) has strong positive synergistic effect whereas Lemon oil (B) has weak positive synergistic effect on release of drug in PBS (at pH 6.8). The combined impact of two factors (AB) has negative antagonistic effect. The quadratic contribution of Rose oil (A^2) has strong negative antagonistic effect while Lemon oil (B^2) has weak negative antagonistic effect. From this equation, the two terms having Rose oil (A) [$38.28A-30.68A^2$] revealed that % drug release increased by increasing the amount of Rose oil and decreased by decreasing the amount of Rose oil. In the similar way, two terms having Lemon oil (B) [$26.35B-4.07B^2$] revealed that percentage drug release increased by increasing the amount and decreased by decreasing the amount of Lemon oil. The contour plots and 3D surface plots are shown in figure 4. The multiple linear regression analysis of response was evaluated for the calculation of comparative values including % Co-efficient of variation, F-value, adjusted R^2, P-value, PRESS, and mean ± SD. The experimental outcomes from ANOVA, contour and 3D surface plots has shown the influence of lemon oil in the release of linseed oil from emulgel formulations as at increased concentration of lemon oil, the release of linseed oil also increased.

Optimization of Linseed oil emulgel formulations

There was comparatively difference in drug release profile from all linseed oil emulgel formulations through cellophane membrane within 24 h time period. The results deducted from RSM data analysis, contour and 3D surface plots indicating EG6 (containing 0.75% rose oil and 1.8% lemon oil) has the maximum % drug release (96.69%) at pH 6.8 than all other linseed oil emulgels. It has revealed that linseed oil emulgel EG6 release through cellophane membrane in lesser time and depicted maximum drug release than all other formulations. Therefore, EG6 linseed oil emulgel formulation was optimized and chosen for further investigation *ex-vivo* /*in-vivo* studies in animal/human models to confirm results.

Determination of Sun Protection Factor (SPF)

SPF value up to 20 is considered to be best for skin. The results has shown that all linseed oil emulgel formulations having SPF value were in range of 14.56 ± 0.01 to 19.9 ± 0.01 and has

considered best for skin protection from ultraviolet rays. The optimized formulation has shown satisfied SPF value in accordance with previous reports.[36-38]

Antibacterial activity

The result for antibacterial activity of optimized EG6 was 85% inhibition that confirmed its antibacterial effectiveness to skin against microbes. The optimized emulgel has strong antibacterial and antimicrobial activities, so considered safe for transdermal use. The similar findings have been reported in previous studies of herbal oils.[40-43] Linseed oil is a source of polyunsaturated fatty acids such as alpha-linoleic acid and related chemicals in linseed oil seem to decrease inflammation.[39-41] Lemon oil and rose oil have also good analgesic and anti-inflammatory activity due to the presence of vitamin C.[42-44] That is why optimized Linseed oil emulgel formulation has thought to be useful for rheumatoid arthritis and other inflammatory (swelling) diseases as literature supported.[45,46]

Accelerated stability studies

Accelerated stability studies of all prepared linseed oil emulgels revealed that all emulgels were stable and has shown no proper significant changes in pH, consistency, %drug content and homogeneity. Only there is a slight change in color of some formulations but it did not effect on their pH, consistency, % drug content and homogeneity.

CONCLUSION

The above results support that formulated emulgel from natural source is new innovation and an alternative to conventional topical preparations as the combination of linseed oil, lemon oil and rose oil has shown strong antibacterial activity, suitable SPF for skin to protect against UV rays and provide smooth texture to skin with lustrous and cleansing effect. Moreover, the stability study has shown no significant effect on the viscosity, homogeneity and pH of all emulgel formulations. In summary, linseed oil emulgel formulation has fulfilled the pharmaceutical requirements and considered safe for skin use.

Conflict of interest

The authors declare that there is no potential conflict of interest associated with this study.

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Approval by Ethical Committee

The approval for *Ex-Vivo* studies in animals and human were taken from the "Ethical Committee " of Faculty of Pharmacy, B.Z.University Multan under the refrence number 1205/PEC/2018.

Availability of data and materials

The materials were obtained from different sources as mentioned under heading 'materials'.

Declaration of interest

The authors declare that there are no potential conflicts of interest associated with this study.

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List of abbreviations

E: Emulgel

RSM: Response surface methodology

ANOVA: Analysis of variance

MLRA: Multiple linear regression analysis

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