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Formulation development and evaluation of transdermal emulgel using Aloevera extract and natural penetration enhancers

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Abstract: This study was to formulate Aloevera extract loaded emulsion (O/W) based gels, by using various concentrations of rose oil, olive oil and Lemon oil as natural penetration enhancers for transdermal effect to treat skin problems. By using RSM, Aloevera emulgels were formulated and then optimized. Stability studies, physico-chemical characteristics, spreadability, skin protection factor, thermal analysis, FTIR, antimicrobial activity, *in vitro* drug release study (at 37ºC with 100 rpm for 180 minute in release medium at pH 5.5) and *in vivo* skin evaluation tests were performed. The results were then statistically analyzed. Among all formulations, G12 has shown maximum 93.53% Aloevera release at higher concentration of Olive oil with decreased concentration of Rose oil and Lemon oil. Analysis of variance (ANOVA) was conducted to evaluate the results exhibited independent variables have remarkable effects on dependent variables. Contour plot is also drawn to express the response between independent and dependent variables. All formulations have followed Korsmeyer-Peppas kinetic model. In summary, the combination of penetration enhancers in Aloevera emulgel can be successfully utilized for treatment of mild-moderate acne vulgaris and other skin problems, as optimized emulgel has shown good permeability, prolonged residence time on skin surface and proved good anti-microbial activity.

Keywords: Aloevera, emulgel, response surface methodology, SPF, antimicrobial activity, drug release kinetics.

INTRODUCTION

Due to convenience and affordability, topical routes have advantages for local delivery of drugs into various regions like skin, nose and eyes etc. (Barry, 2001, Yadav, 2012) However these routes are challenging to provide optimal concentration of drug at its action site. Because various defensive barriers, for example, stratum corneum affect the delivery of drug into skin. To overcome this situation, new topical drug delivery systems are developed. One of them is emulgel. It is one of the recent technology in NDDS used topically having properties of dual control release i.e. emulsion as well as gel (Yadav *et al*., 2016). They can be water in oil or oil in water type emulsion that is gelled after mixing with gelling agent. (Ashara *et al*., 2017) It enhances the permeability of drug into skin due to the use of emulsifiers, gelling agents and broad range of carrier molecules. Scientists believe that it shows better release of active ingredient than other topical route. So cosmetics point of view, emulgels are gaining attention due to non-greasy, good spreadability, pleasant appearance, water solubility and easily absorbed. (Sintov and Botner, 2006)

Now-a-days in cosmetics, natural herbs having anti-aging property, anti-acne, anti-inflammatory as well as antioxidant property and moisturizing property for skin (Rajvanshi *et al*., 2011) are incorporated into emulgel to overcome the side effects of synthetic drugs.

Aloevera is extremely beneficial for showing these properties. It consists of 75 active ingredients, i.e. vitamins, minerals, sugars, salicylic acids, lignin, saponins and amino acids. Aloe vera has a power to penetrate deeply into dermis layer of skin. So it helps in retaining moisture in damaged tissue (Pegu and Sharma, 2019).

The aim of my research was to formulate Aloevera emulgel due to above mentioned properties. Sixteen different batches (G1, G2, G16) of Aloevera emulgel were designed using different concentration of penetration enhancers (Lemon oil, Rose oil and olive oil) for optimization through Response surface methodology (RSM).While concentration of permeation enhancer (propylene glycol) and polymer (Carbopol-940)were kept constant (Levang *et al*., 1999). To investigate drug release kinetics from these formulated emulgels, drug release studies were conducted through cellophane membrane in PBS at pH 5.5 using USP dissolution apparatus (Dissolution Tester).

MATERIALS AND METHODS

Materials

Aloevera Leaf extract (from plant), Rose oil, Olive oil and

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Lemon oil (from local market), Propylene glycol, Carbopol-940 polymer, Tween20, Span20, Triethanolamine, Methyl paraben, Methanol (all of Merck German origin and purchased from local market). Double Distilled Water was obtained from distillation apparatus of Pharmacy department, B.Z. University, Multan.

Preparation and evaluation of Aloevera extract in Phosphate buffer solution (at pH 5.5)

Preparation of buffer solution was achieved by using KH_2PO_4 and NaOH. Then calibrated for its pH (5.5) by pH meter. 10mg of Aloevera extract was dissolved in 100ml phosphate buffer solution (pH 5.5) to make stock solution and then different dilutions were prepared having concentration in range of 1-5µg/ml. These were then analyzed by UV Spectrophotometer (Perkin Elmer Lambda 25) for its absorbance at 300nm.Then calibration curve was drawn, having regression equation Y= 0.0053x + 0.2433with regression coefficient 0.9933; where Y = absorbance at 300nm, m = slope, b = intercept and X = concentration (µg /mL).

Preparation of Aloevera emulgels

Aloevera emulgels were formulated by using varying concentrations of penetration enhancing agents (Lemon oil, Olive oil and Rose oil) and surfactants (Tween 20 and Span 20) according to RSM as shown in table 1. For Aloe vera emulgels preparation, weight of each ingredient was adjusted according to their respected HLB (Hydrophilic - Lyophilic Balance ratio). (Americas, 1984, Griffin, 1955, Griffin, 1949) The gel phase was prepared by mixing the required amount of carbopol-940 in sufficient quantity of distilled water with continuous stirring via the help of magnetic stirrer till no lumps. To make an oil phase, span 20 was added in already mixed oils i.e. Rose oil, Olive oil and Lemon oil (penetration enhancers) on continuous stirring. Aqueous phase of emulsion was formulated by mixing Tween 20 in small amount of distilled water. Then Aloevera extract was added in it on continuous stirring. In required amount of Propylene Glycol (permeation enhancer), diluted methyl paraben was added as preservative and then mixed this solution with aqueous phase on continuous stirring. Both oil and aqueous phases were heated separately at 70-80ºC for 10 minutes and then cooled at room temperature. After cooling, oil phase was added gradually in aqueous phase on continuous stirring to make O/W emulsion (having HLB value 4.5-5.5). It is mandatory to combine these two phases at room temperature as high temperature causes the droplets of oil to coalesce and very low temperature cause them to freeze. Then mix this O/W emulsion into gel phase on continuous stirring through use of magnetic stirrer. pH was adjusted at 5.5 by adding Triethanolamine drop wise and then adjusted the required weight by adding distilled water on continuous stirring until required consistency was attained. These all formulations were kept and stored in aluminum collapsible tubes for further evaluation.

Physical evaluation of Aloevera emulgel

Physical evaluation of all Aloevera emulgel formulations including homogeneity, transparency, viscosity, emulgel texture, drug content and pH were inspected (Contreras and Sanchez, 2002).

pH determination

The pH of all formulations were determined by pH meter (Mettler & Toledo, Giessen, Germany) at room temperature (25±0.5°C).

Rheological study

The viscosity of all formulations were examined through digital Brookfield viscometer (Brookfield engineering labs, model rvtdv 11, Inc., Stoughton) carrying spindle 63 at room temperature (25±0.5°C).

Visual appearance

Each formulation was analysed by naked eye for its uniformity, change in colour, texture, phase separation and presence of any lumps.

% drug yield and drug content uniformity

The % drug yield of all formulations were calculated by measuring the theoretical mass at the time of formulation preparation and practical mass after 24 hours by use of following formula (Wang and Guo, 2008);

$$\% \text{ Drug yield} = \text{Practical mass} / \text{Theoretical mass} \times 100 \quad (1)$$

The drug content in each Aloevera emulgel formulation was determined by adding 1gm of each formulation in 100ml emulgel phosphate buffer solution (at pH 5.5) and shake it well. After filtration, appropriate dilution was made and analyzed through UV Spectrophotometer at 300nm at room temperature (25±0.5°C). Repeat the same procedure with blank sample (without drug) and determined the drug content by following formula;

Drug content= Absorbance of blank/Absorbance of sample containing drug x 100 (2)

Spreadability

The spreadability of each formulation was determined by putting the 1gm formulation in 2cm circle between two glass slides (15 x 10.5 cm and 5mm) slides (Helal *et al*., 2012), keeping the 5gm weight on the upper glass slide for 5 minute (Abdel-Mottaleb *et al*., 2007). After this, measured the diameter (in cm) of spreader emulgel in circular area (Abd El Gawad, 2014) and calculate it by using following formula;

$$S = M \times L / T \quad (3)$$

Whereas; S is for spread ability, M is for mass of weight (gm), L is for length of spreader 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°C).

Extrudibility

Extrudibility is the measurement of ability of emulgels flow from collapsible tube. For this test, 20gm of each emulgel formulation was filled in aluminum collapsible tubes and placed 1kg weight on the flat surface at the closed end of tube for 30 seconds. The quantity of extruded emulgel from each tube was noted and calculated the extrudibility (extrusion pressure/gram) (Chakole *et al*., 2009).

Determination of Aloevera extract Solubility and partition coefficient (Ko/w)

The solubility of Aloevera extract was determined in methanol and phosphate buffer saline (at pH 5.5). The excessive quantity of Aloevera was added to each solvent (5ml) under controlled thermostatically stirring at 37^{o}C for 48 days. Later on, each solution was kept for centrifugation at 13000 RPM for 15 minutes. The supernatant aliquot was separated and after appropriate dilution, the solvent was analyzed through UV Spectrophotometer at 300nm. The solubility of Aloevera in each solvent was determined in triplicate at room temperature (25±0.5°C) and results were calculated as mean ± SD.

The small quantity of Aloevera was mixed in distilled water (5mL) in 100mL Separating Funnel and then shaken till 15 minutes. After this, octanol (5ml) was added in this and shaken for further 5 minutes and allowed to keep it for 24 hrs. The supernatant aliquot was collected and centrifuged at 13000 rpm for 5 minutes. Each solvent was evaluated at 290nm through UV Spectrophotometer after appropriate dilutions. The solubility of Aloevera was determined in both of these solvents and calculate its partition coefficient in octanol/water. Repeat this experiment in triplicate at room temperature (25±0.5°C) for accuracy.

Experimental design

For optimization of Aloevera emulgel, response surface methodology is achieved. To attain this methodology, Design Expert [trial version 7.3, State-Ease Inc. Minneapolis, MN and USA] using CCD (central composite design) considering (α=2) as per standard protocol is utilized. The amounts of lemon oil, olive oil and Rose oil were selected as numeric factors and studied at three levels each. The central point (0, 0) was studied in quintuplicate (Ghica *et al*., 2011, Chang *et al*., 2007, Shiyani *et al*., 2008). The remaining formulation variables were kept invariant in whole study.

In vitro evaluation of Aloevera emulgel

Evaluation of all prepared emulgels were done under following parameters.

Fourier transforms infrared spectroscopy (FT-IR)

The interaction between drug and polymer is observed via FT-IR by using Disc method (KBr). FT-IR spectrum was scanned and recorded. (Kaushik *et al*., 2013)

Thermal analysis

Thermogravimetric (TGA) study was performed on optimized Aloevera emulgel. Put 100mg sample in the aluminum pans, by switching the gas to Nitrogen at 20.0 ml/minute. Then kept for 1.0 min at 40.0°C and heated from 40-200°C in nitrogen atmosphere at 10°C/ min by Perkin-Elmer thermal analysis. Thermograms peak was recorded.(Kaushik *et al*., 2013)

Drug release study

In-vitro drug release study of all formulated emulgels was carried out in dissolution apparatus (USP type-II) by keeping 1gm each emulgel in cellophane membrane, tied firmly with paddle, using 500ml release medium of PBS (pH 5.5) at 37±2°C on constant stirring 100 RPM (Badshah *et al*., 2012).

After specific time intervals (0, 5, 10,15,30,45,60,120, 150,180 min), sample was drawn, replaced by fresh sample in dissolution apparatus, and then analyzed for absorbance at 300nm through UV spectrophotometer. The % drug release of Aloevera in each emulgel formulation was calculated by using regression equation. The same procedure was repeated in triplicate for more accurate results.

Drug release kinetics

The fate of Aloevera release was determined by fitting release data to five kinetic models (zero order, first order, Higuchi ,Hixson Crowell, Korsmeyer-Peppas model) through DD Solver (version 10) (Zhang *et al*., 2010, Shah *et al*., 2013). For validity of best fit model among all of these mentioned models, AIC (Akaike information criterion) was applied, using DD Solver (Obata *et al*., 2010).

Ethical approval

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 184/PEC/2019.

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 16 different formulations were determined by using the regression analysis and one-way analysis of variance (ANOVA) with $p < 0.05$ as a minimal level of significance (Akaike, 1974).

RSM Optimization data

In this present research, computerized based streamline method with RSM usage of polynomial equation has been attained (Shah *et al*., 2009). Polynomial equations with the interaction and quadratic terms were generated for

response Y (% drug release in PBS at pH 5.5) by use of Multiple Linear Regression Analysis (MLRA) approach. The total % amount of Aloe vera was drawn as a function of time. Contour plots and 3D images were plotted to select the formulation variables require to generate desired value.

Determination of Sun Protection Factor (SPF)

Each emulgel weighing 1gm was introduce in 100 ml ethanol and then ultra-sonicated for 10 minutes. After filtration, 5ml of aliquot was shifted in 50 ml volumetric flask and diluted it with ethanol up to mark. Again filtered it and 5ml of aliquot was shifted to 25ml volumetric flask and diluted it with ethanol up to mark. To determine the Sun protection factor, a mathematical equation was generated which put in vitro method using UV spectrophotometry (Mansur *et al*., 1986, Sayre *et al*., 1979) by measuring absorbance at 290-320±5nm, against ethanol as reference.

$$\text{SPF spectrophotometric} = \text{CF x } 320\Sigma 290 EF(\lambda) \times I(\lambda) \times Abs(\lambda) \quad (4)$$

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 (Steiger, 2010).

Antibacterial activity

Ditch plate technique is used to evaluate the antibacterial activity of optimized emulgel. Nutrient agar media was utilized to see the bactericidal/bacteriostatic activity (Staphylococcus epidermidis). Took the fresh pus from face acne of any volunteer via the cotton and applied this pus via the sterilized loop onto agar plate. After 24 hrs at 25ºC±0.5, observed the bacterial growth at these plates. Then optimized emulgel (1gm) on this agar plate was added on. Now streak over the agar at right angle to all over the plate and incubate for 24hrs at 25ºC±0.5. By using crystal violet dye, microbial growth under microscope was evaluated. Length of inhibition was measured and then calculated the % Inhibition (Steiger, 2010).

% inhibition = Total length of inhibition (L2) / Total length of Streaked culture (L1) x 100 (5)

Anti- inflammatory test

Patient with allergy (small red bumps on arm due to insect bites) was treated with Aloe Vera emulgel thrice a day. After 2 days, red spots disappeared completely.

Accelerated stability test

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

In vivo characterization

Anti-acne test

A 35-year-old female patient suffering from mild-moderate acne on face was treated with Aloe Vera emulgel. She was instructed to apply emulgel twice a day with sunblock. After 3 months, acne problem resolved.

Patch test

It was performed to look for any irritation or allergic reaction of formulation on forearm of each volunteer. For single application of formulation, closed patch test (24hrs), area (5cm x 4cm) was marked on the inner right forearms of all female volunteers in a group (10). A small amount of formulation applied on this marked area. This area was covered with occlusive cotton bandage, fixed with adhesive tape.

Determination of Skin Moisture Contents with Corneometer® CM 825

With the help of Corneometer, moisture contents of human skin were measured before the application of active formulation and then after every second week for a period of three months. Moisture content should be high in dermatology and cosmetic point of view. The measurement of skin moisture content is dependent on the internationally adopted Corneometer method.

SELS Determination with Visioscan® VC 98

Four parameters for Surface Evaluation of Living Skin (SELS) were measured. These are Surface evaluation of roughness (SEr), Surface evaluation of scaliness (SEsc), Surface evaluation of smoothness (SEsm), Surface evaluation of wrinkles (SEwr). These evaluations were done at zero time and then after first, second, third month of study period by the use of Visioscan® VC 98. The basic principle of SELS is based on the evaluation of living skin image taken under certain illuminations. The picture obtained is electronically processed quantitative results. These all above mentioned four parameters are evaluated by grey level distribution of the image as an index. If the value of surface evaluation of roughness is small, then it shows the more smoothness and if it is large, it indicates the more roughness. Surface evaluation of scaliness evaluates the hydration of stratum corneum. If its value is small, it shows more hydration and vice versa. Similarly, high value of surface evaluation of smoothness shows more smoothness and high value of surface evaluation of wrinkles show more wrinkles. These parameters were measured before application of formulation (zero time), and then after first, second and third months of study period.

Panel Test

A form for panel test was given to each volunteer. It consisted of following questions:

1. Spreadability
2. Ease of application

Parameters	Rating points
Ease of application	5(best to apply)
Decrease of oiliness	5(feel no oil after application)
Sense in long term	5(hydrate the skin for long time)
Sense just after application	5(skin becomes softer and smoother)
Irritation	1(no allergy)
Shine on skin	4(give better look)
Sense of softness	4(skin looks soft and younger)
Decrease acne	5(acne reduced gradually)
Sense of Sun protection	4(Brown spots and acne spots decreased gradually)

3. Sense immediately after the application
4. Sense in long term use
5. Sense of smoothness
6. Shines on skin
7. Irritation on skin

These seven parameters were evaluated in this panel. Each of these assigned eleven values from -5 to +5 showing very bad to very good values, with 0 as baseline respectively. This form was completed by each human volunteer after twelve weeks of study period independently.

RESULTS

Determination of Aloevera Solubility and partition coefficient (Ko/w)

The results of Aloevera solubility is methanol<phosphate buffer (at pH 5.5). The solubility of Aloe Vera in 0.00185±0.45mg/ml in PBS, 0.00084±0.67 mg/ml in Methanol.

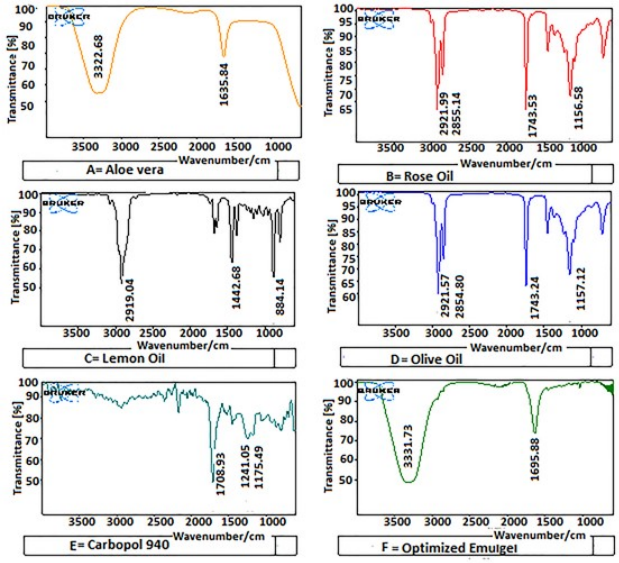

Fig. 1: FT-IR peaks of A= Aloe vera ,B= Rose Oil,C= Olive oil, D= Lemon oil, E= polymer, F= Optimized emulgel (G12)

The Partition coefficient (Ko/w) for Aloevera 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 (Iman *et al*., 2010).

Physical evaluation of Aloevera emulgel formulations

Physical characteristics like homogeneity, texture (greasy/non-greasy), pH, phase separation, viscosity and smoothness of all prepared Aloevera emulgel were observed.

Results have shown that all formulations were smooth, good homogeneity, transparent, non-greasy and lumps free. pH value of was in range of 5.4-5.7±0.1, so considered suitable for skin application. All formulations have good consistency with the viscosity in range of 640-671*10^3 (cps).

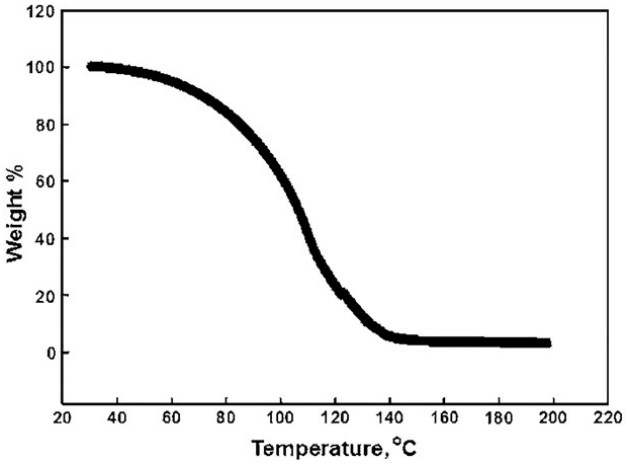

Fig. 2: Thermal analysis of optimized Aloe vera emulgel (G12)

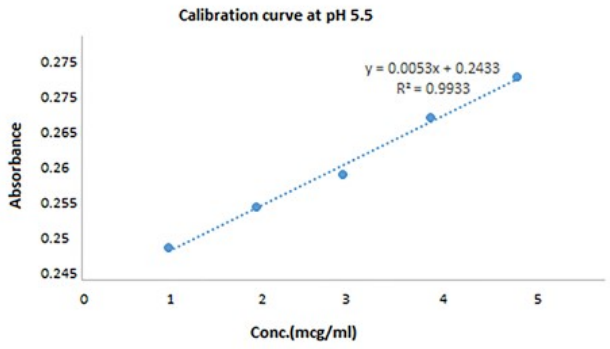

Fig. 3: Calibration curve at pH 5.5

Spreadability value of all emulgel formulations was in range of 0.034±0.1 to 0.046±0.1g.cm/s while extrudibility value of these formulations were in range of 0.95±0.01 to 1.31±0.01g/cm. Both these parameters have depicted that G12 easily spread (0.042±0.1g/cm) after applying small amount of the shear stress and having good extrudibility value (1.27±0.01), though proving its excellent consistency as compared to others.

Determination of % drug yield and drug contentment uniformity test

This study was done to examine the % drug yield of all prepared emulgels for six months' time period, indicating that % drug yield was in range of 95.5±0.2 to 99.6±0.1 whereas drug content of all formulations were in range of 94.8±0.1 to 99.6±0.1.

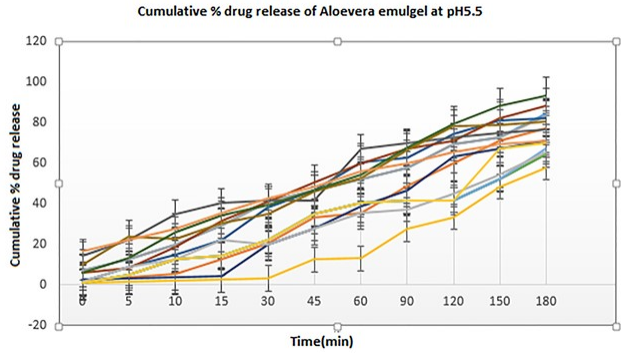

Fig. 4: Cumulative % Drug release profile of Aloe vera emulgel formulations at pH 5.5

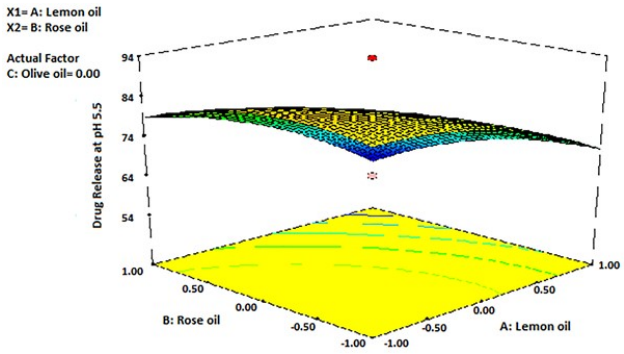

Fig. 5: 3D surface plot of % drug release at Y (pH 5.5)

Evaluation of Aloevera emulgel formulations

Fourier Transform Infrared spectroscopy (FT- IR)

The FTIR spectra in fig. 1 has shown no significant difference in polymer (carbopol-940), pure Aloevera, Rose oil, Lemon oil, Olive oil and optimized Aloevera emulgel (G12). The peaks in range of 2800-3400/cm was due to alkane group (-CH3) and these were very sharp in all spectrum except polymer because of the coordination of linkages. Some peaks were seen in range of 1600 - 1800/cm were due to the alkene group (C=C) and this was sharper in polymer and olive oil spectra as compared to others. This has been indicating strong bond interaction among alkene group of polymer and olive oil. Whereas, peaks in the range of 1020-1175/cm were due presence of phenyl group. Results of FTIR spectra of Aloevera were found to be in good agreement and suggested the Aloevera stability in emulgel formulation with respect to carbopol-940.

Thermal analysis

The stability of Aloevera in Carbopol 940 was investigated by thermal analysis using TGA thermograms. The melting point of drug loaded optimized G12 emulgel of Aloevera was revealed between 60-140ºC. The loading temperature was 40ºC. The result of thermal analysis proved the stability of this Aloevera emulgel at molecular level. The TGA curve of optimized emulgel G12is shown in fig. 2.

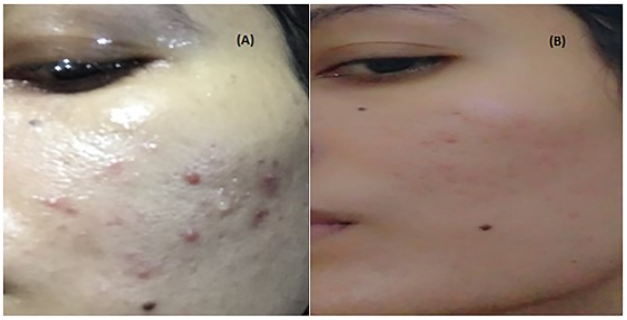

Fig. 6: Anti acne activity of optimized emulgel

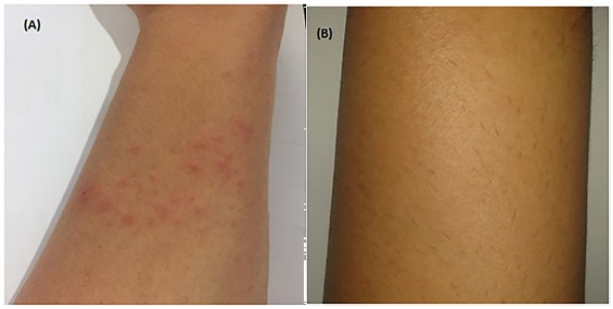

Fig. 7: Anti-inflammatory activity of optimized emulgel

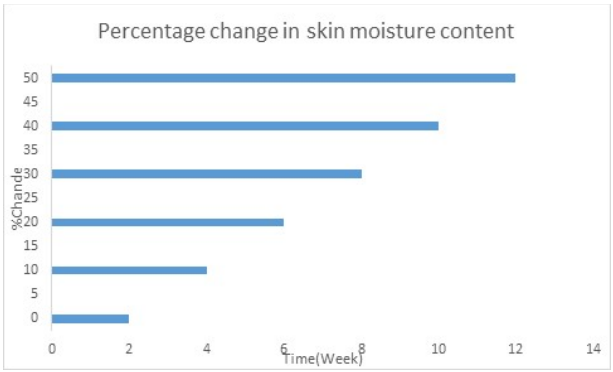

Fig. 8: Percentage change in skin moisture content with respect to time

In vitro drug release study

The release of Aloevera from all emulgel formulations was analyzed for 180 min. The release amount was calculated by using regression equation for calibration curve y = 0.0053x+0.2433 with regression coefficient R^2

= 0.9933 at pH 5.5. The results indicated that formulated Aloevera emulgel G12 has shown the highest drug release (93.53%±0.01). Calibration curve and cumulative % drug release profile of all Aloevera emulgel formulations at pH 5.5 (n=3±SD) has shown in table 2 and figs. 3 and 4.

Data analysis

The statistical data analysis and one-way ANOVA were carried out using these absorbance values by the help of Microsoft excel, version 13. The significant statistical differences among sixteen formulated emulgels were observed by Regression analysis and one-way ANOVA (analysis of variance). The minimum level of significance was at $p<0.05$.

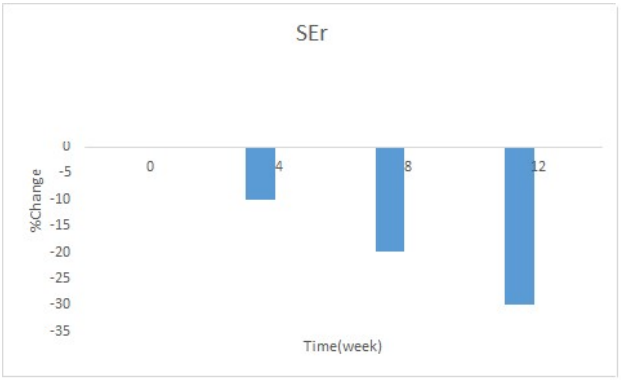

Fig. 9: Percentage change in surface evaluation of skin roughness after applying optimized emulgel

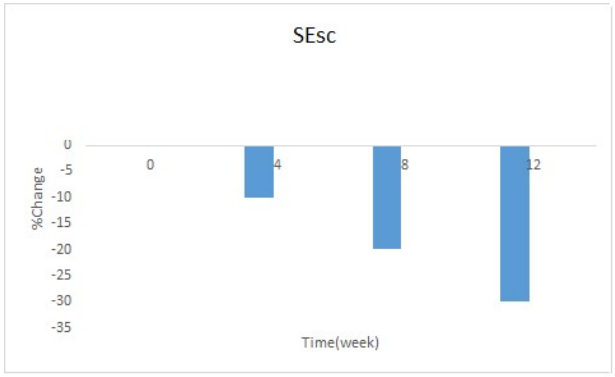

Fig. 10: Percentage change in surface evaluation of skin scaliness after applying optimized emulgel

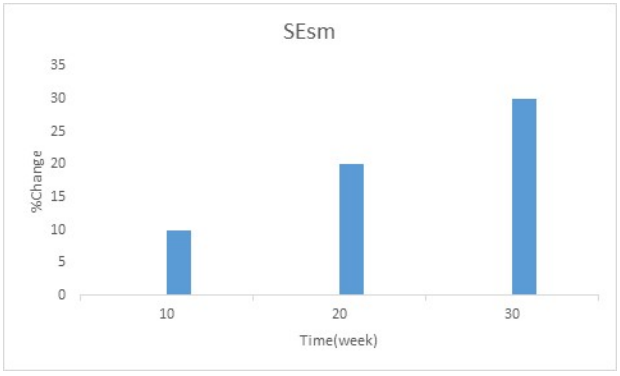

Fig. 11: Percentage changes in surface evaluation of smoothness after applying optimized emulgel

Drug release kinetics

The mode of drug release of prepared emulgels has depicted that Korsemeyer-peppas model was the most favorable model for all prepared emulgels of Aloevera at 5.5 pH due to the greatest coefficient of determination value (R^2) and lowest AIC value as shown in table 3.

RSM Optimization data modeling

For creating a mathematical relationship, multiple linear regression analysis was used i.e. expressed as polynomial equation. The positive values of coefficient represent synergistically effect while negative values illustrate antagonistically effect on response. The higher value of coefficient depicts that the factor has the powerful impact upon response. The result of Multiple Linear Regression Analysis of response has shown % Co-efficient of variation (26.11%), F-value (1.72), R^2 (0.72) and mean ± SD (75.86 ± 9.45).

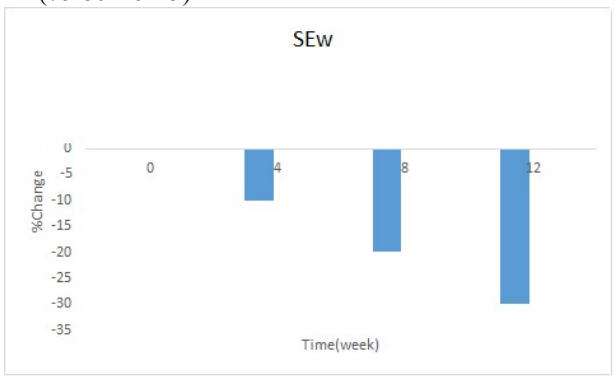

Fig. 12: Percentage changes in surface evaluation of wrinkles after applying of optimized emulgel

Effect of enhancers on % drug release at Y (pH 5.5)

Non significance probability P value ($p>0.05$) for response Y depicts that linear participation Rose oil (B) and Olive oil (C) has produced non-significant effect ($p> 0.05$) synergistically while Lemon oil(A) has produced Significant effect ($P<0.05$) synergistically. On the other hand, the cross product participation of Rose oil and Lemon oil (AB), Lemon oil and Olive oil(AC) produced non-significant effect ($p>0.05$) antagonistically while Rose oil and Olive oil (BC) produced non-significant effect ($p>0.05$) synergistically. Quadratic contribution A^2 and B^2 produce non-significant ($p>0.05$) synergistic effect while C^2 produce non-significant ($p>0.05$) antagonistic effect. This has been shown in ANOVA table 4.

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

$Y1=73.87+6.57A+4.01B+4.55C-0.11AB-1.46AC+4.09BC+0.19\ A^2+3.05B^2-\ 0.92C^2$

The above equation depicts that Lemon oil (A) has strong positive synergistic effect whereas Rose oil (B) and Olive oil(C) has weak positive synergistic effect on release of drug in PBS (at pH 5.5). The combined impact of factors (AB) and (AC) has negative antagonistic effect while

Table 1: Factors combinations as per (a) chosen experimental design & (b) translation of coded levels in actual units

Trial no	Coded factor levels			Rose oil	Lemon oil	Olive oil
	$X_{1(Rose\ oil)}$	$X_{2(Lemon\ oil)}$	$X_{3(Olive\ oil)}$			
G1	0	0	1	1.65	1.65	6.2
G2	0	1	0	1.65	2.24	4.1
G3	1	1	-1	2	2	2
G4	0	0	-1	1.65	1.65	2
G5	-1	-1	-1	1.3	1.3	2
G6	-1	-1	1	1.3	1.3	6.2
G7	1	-1	-1	2	1.3	2
G8	-1	1	-1	1.3	2	2
G9	1	-1	1	2	1.3	6.2
G10	-1	1	1	1.3	2	6.2
G11	1	1	1	2	2	6.2
G12	1	0	0	2.24	1.65	4.1
G13	0	0	0	1.65	1.65	4.1
G14	0	0	0	1.65	1.65	4.1
G15	0	0	0	1.65	1.65	4.1
G16	0	0	0	1.65	1.65	4.1

Code level	-1	0	1
$X_{1(Rose\ oil)gm}$	1.3	1.65	2
$X_{2(Lemon\ oil)gm}$	1.3	1.65	2
$X_{3(Olive\ oil)gm}$	2	4.1	6.2

Table 2: Cumulative % drug release profile of all Aloe vera emulgels at pH 5.5 (n=3±SD)

TIME(min)	0	5	10	15	30	45	60	90	120	150	180
G1	6.92	12.6	20.13	29.57	40.89	46.55	52.2	57.9	69.2	73	84.28
G2	1.45	3.72	5.604	12.77	20.51	33.34	35.4	48.6	59.8	71.3	77.3
G3	6.92	12.6	20.13	29.57	40.89	46.55	52.2	57.9	69.2	73	83
G4	1.07	1.26	1.83	2.386	3.15	12.58	13	27.7	33.3	48.4	57.86
G5	1.06	4.66	12.58	14.28	22.01	35.04	40.7	41.5	41.8	52.2	64.2
G6	1.06	4.66	12.58	14.28	22.01	35.04	40.7	41.5	41.8	52.2	59.7
G7	1.06	4.66	12.58	14.28	22.01	35.04	40.7	41.5	41.8	52.2	67.3
G8	6.17	8.05	18.8	31.45	40.88	50.3	59.8	67.3	71.1	82.4	88.06
G9	14.3	22	35.04	40.69	41.45	41.83	67.3	70	73	74.8	76.74
G10	9.75	23.9	22.58	30.51	34.66	46.17	52.8	66.7	78.4	78.6	80.5
G11	1.06	4.66	12.58	14.28	22.01	35.04	40.7	41.5	41.8	67.3	70
G12	6.17	13.2	26.17	34.28	39.38	46.55	54.3	67.5	79.2	88.1	93.53
G13	16.4	22	27.67	35.22	20.13	27.67	35.2	37.1	44.7	54.1	65.42
G14	16.4	22	27.67	35.22	20.13	27.67	35.2	37.1	44.7	54.1	65.42
G15	16.4	22	27.67	35.22	20.13	27.67	35.2	37.1	44.7	54.1	65.42
G16	16.4	22	27.67	35.22	20.13	27.67	35.2	37.1	44.7	54.1	65.42

Table 3: Combined values of best (Korsmeyer peppas) of all emulgel formulations

Formulations	R^2	kKP	N	AIC
G1	.98	8.270	.443	56.5143
G2	.99	1.807	.729	47.4337
G3	.98	8.397	.439	56.1512
G4	.99	.090	1.248	43.5302
G5	.96	3.905	.528	61.1237
G6	.94	3.907	.518	60.1234
G7	.95	3.671	.544	62.7408
G8	.97	8.029	.466	63.1076
G9	.89	15.723	.315	72.7842
G10	.96	9.582	.421	64.0626
G11	.95	2.946	.606	65.25
G12	.98	8.005	.475	54.8392
G13	.97	3.547	.546	55.9493
G14	.97	3.547	.546	55.9493
G15	.97	3.547	.546	55.9493
G16	.97	3.547	.546	55.9493

Table 4: Analysis of variance (ANOVA) of Aloe vera emulgel for repose Y

Source	Sum of Squares	df	Mean Square	F-Value	P-Value Prob>F	
Model	1386.42	9	154.05	1.72	0.2611	Non-Significant
A-lemon oil	589.91	1	589.91	6.6	0.0424	Significant
B-rose oil	219.12	1	219.12	2.45	.1684	Non-significant
C-olive oil	282.95	1	282.95	3.17	0.1254	Non-significant
AB	.095	1	0.095	1.06	.9751	
AC	17.02	1	17.02	.19	.6777	
BC	133.91	1	133.91	1.5	.2667	
A^2	0.33	1	.33	3.73	.9533	
B^2	86.37	1	86.37	.97	.3635	
C	7.79	1	7.79	.087	.7777	
Residual	536.06	6	89.34			
Lack of fit	392.07	5	78.41	0.54	0.7666	Non-significant
Pure error	143.99	1	143.99			

(BC) has positive synergistic effect. The quadratic contribution of Lemon oil (A^2) and Rose oil (B^2) has strong positive synergistic effect while Olive oil (C^2) has weak negative antagonistic effect. From this equation, it was shown that % drug release increased by increasing the amount of Lemon oil, Rose oil and Olive oil. The 3D surface plot is shown in fig. 5.

Optimization of Aloevera emulgel formulations

There was the comparatively difference in drug release profile from all emulgel formulations through cellophane membrane within 180 minutes time period. The results analyzed from RSM data analysis, contour and 3D surface plots showing G12 (containing 2.24% Rose oil, 4.1%Olive oil and 1.65% Lemon oil) has the maximum % drug release (93.53%) at pH 5.5 than all other emulgels. It has indicated that Aloevera emulgel G12 release through cellophane membrane in lesser time and showed highest drug release than all other remaining formulations. Therefore, G12 emulgel formulation was optimized and selected for further evaluation *ex-vivo* /*in-vivo* studies in animal/human models to validate the results.

Determination of Sun Protection Factor (SPF)

SPF value up to 20 is considered to be ideal for face skin. The results have shown that all Aloevera emulgel formulations having SPF value were in range of 15.56 ± 0.01 to 19.8 ± 0.01. So, it was considered best for skin protection from ultraviolet rays.

Antibacterial activity

The result for antibacterial activity of optimized G12 was 85% inhibition that confirmed its antibacterial efficacy to skin against microbial growth.

Accelerated stability studies

Accelerated stability studies of all prepared Aloe vera emulgels indicated that all emulgels were shown best stability. There were no proper significant variations in pH, consistency, %drug content and homogeneity. Only there was slightly color change in some formulations but it did not effect on their pH, consistency, % drug content

In vivo Evaluation

Anti-acne test

The decrease in acne formation is observed on skin of acne patient. It shows the anti-acne property of emulgel. Results were shown in fig. 6.

Anti- inflammatory test

After one day of emulgel application, the insect bite red spots were reasonably vanished and totally finished after second day of application. Results were shown in fig. 7.

Patch test (Skin irritation assessment)

The optimized Aloevera emulgel were applied on the forearm of 10 healthy volunteers from the university of Bahauddin Zakariya University, Multan. These were applied daily for the seven days for 24 hours and results (skin irritation/lesion/abrasion) were examined after each day and shown absence of skin lesion or abrasion.

Moisture content for optimized emulgel

Percentage of changes in skin moisture content after the application of optimized formulation of G12 on human female volunteer's skin were noted at the base line and then after every second week for a study period of twelve weeks. Results were shown in fig. 8. All values were taken in triplicates (n=3). Improvement in skin moisture of skin layer (stratum corneum) provides a younger and smoother look [34]. The presence of high moisture content in this formulation is due to high moisture index of Aloevera i.e. about 99%.

Surface Evaluation of Living Skin (SELS)

SELS parameters give assessment of the structure of the skin surface including roughness, scaling smoothness and wrinkling. Effect of optimized emulgel on ten healthy female volunteers were observed according to these four parameters. Improvement in these parameters after topical

application of optimized emulgel was due to the presence of antioxidants in it.

Surface Evaluation of Roughness (SEr) for optimized formulation

Percentage of changes in SEr were measured after the application of active formulation for three months at zero hour, first month, second month and third month of study period. Results have been shown in fig. 9. All values were noted in triplicate (n=3). It was noted that skin roughness parameter was improved after the topical application of optimized emulgel.

Surface Evaluation of Scaliness (SEsc) for optimized formulation

Percentage of changes in SEsc were measured after the application of active formulation for three months at zero hour, first month, second month and third month of study period. Results have been shown in fig. 10. All values were noted in triplicate (n=3). It was assessed that optimized emulgel showed the significant effects as hydration level of skin was improved.

Surface Evaluation of Smoothness (SEsm) for optimized formulation

Percentage of changes in SEsm were measured after the application of active formulation for three months at zero hour, first month, second month and third month of study period. Results have been shown in fig. 11. All values were noted in triplicate (n=3). A gradual increase in smoothness was observed.

Surface evaluation of Wrinkling (SEw) for optimized formulation

Percentage of changes in SEw were measured after the application of active formulation for three months at zero hour, first month, second month and third month of study period. Results have been shown in fig. 12. All values were noted in triplicate (n=3). It was noted that skin wrinkling was reduced.

Panel test

A questionnaire was circulated to evaluate the effectiveness of active formulation among volunteers for a study period of twelve weeks. It was rated from No.1 to No. 5. The no 1 indicated poor quality and no. 5 indicated excellent.

DISCUSSION

The drug contents of all emulgel formulations were increased by increasing the concentration of Lemon oil and Rose oil and at decreased concentration of Olive oil. The similar findings have been reported in previous studies (Fukumoto *et al*., 2006). The drug release profile of all emulgel formulations (G1, G2, G16) at pH 5.5 showed abrupt release of Aloevera from all formulations due to low concentration of Rose oil and Lemon oil and high concentration of olive oil except G12 that showed its maximum release in moderate manner in lesser time having increased concentration of all essential oils. These findings are in accordance with the pervious published reports (Charoo *et al*., 2005, Kaza and Pitchaimani, 2006). The mode of drug release of prepared emulgels has revealed that Korsmeyer peppas model was the most fit model for all prepared emulgels of Aloevera at 5.5 pH due to the greatest coefficient of determination value (R^2) indicating that mode of drug release didn't depend on concentration of drug. 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 and contour plots has revealed the influence of essential oils on the release of Aloevera from emulgel formulations as at increased concentration of Lemon oil and Rose oil, the release of Aloevera also increased. All in-vitro physical evaluation expressed the good homogeneity, transparency, viscosity, extrudibility, spread ability and stability for prolonged time period. The optimized Aloevera emulgel has no skin irritation and has shown excellent results for skin care. The provided results are in accordance with previous reports (Kaza and Pitchaimani, 2006). The optimized formulation has shown acceptable SPF value in accordance with previous reports (Ng *et al*., 2000, Malhotra *et al*., 2009, Pratt and Hudson, 1990). The optimized emulgel has strong antibacterial, antimicrobial and anti-inflammatory activities, so considered safe for transdermal use. The similar findings have been reported in previous studies of herbal oils (Kuljanabhagavad *et al*., 2010, Andoğan *et al*., 2002, Gochev *et al*., 2008, Ulusoy *et al*., 2009). Aloevera is a source of many minerals, vitamins, phenols and salicylic acid which seem to decrease skin inflammation (Surjushe *et al*., 2008). Lemon oil and Rose oil have also good analgesic and anti-inflammatory activity due to the presence of vitamin C (Maleev *et al*., 1972, Tannenbaum *et al*., 1991, Hajhashemi *et al*., 2010) while Olive oil is also effective for decreasing inflammation of skin. As olive oil has phenols which act as antioxidants (Lin *et al*., 2018). Moreover, all dermatological tests on human volunteer confirms that this emulgel is very effective to retain moisture on skin, to reduce scaliness and roughness of skin and to improve smoothness of skin. It also gives shine to skin with reduction in acne. That is why optimized Aloevera emulgel formulation has thought to be super effective for inflammatory diseases of skin.

CONCLUSION

The above result supports that developed Emulgel from natural source is new innovation and an alternative option to conventional topical preparations. In this study, Aloevera extract, lemon oil, olive oil and rose oil

combination has shown strong antibacterial activity, anti-inflammatory activity, SPF suitable for skin to protect against UV rays and provide smooth non greasy texture to skin with lustrous and cleansing effect. Similarly, the stability study has shown no significant effect on the viscosity, homogeneity and pH of all emulgel formulations. So conclusion is that Aloevera emulgel formulation has fulfilled the pharmaceutical requirements and considered safe for skin use.

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