

Formulation Development and Moisturising Effects of a Topical Cream of *Aloe vera* Extract

Akhtar N, Khan BA, Khan MS, Mahmood T, Khan HMS, Iqbal M and Bashir S

Abstract—This study was designed to formulate, pharmaceutically evaluate a topical skin-care cream (w/o emulsion) of Aloe Vera versus its vehicle (Base) as control and determine their effects on Stratum Corneum (SC) water content and Transepidermal water loss (TEWL). Base containing no extract and a Formulation containing 3% concentrated extract of Aloe Vera was developed by entrapping in the inner aqueous phase of w/o emulsion (cream). Lemon oil was incorporated to improve the odor. Both the Base and Formulation were stored at 8°C ±0.1°C (in refrigerator), 25°C±0.1°C, 40°C±0.1°C and 40°C± 0.1°C with 75% RH (in incubator) for a period of 4 weeks to predict their stability. The evaluation parameters consisted of color, smell, type of emulsion, phase separation, electrical conductivity, centrifugation, liquefaction and pH. Both the Base and Formulation were applied to the cheeks of 21 healthy human volunteers for a period of 8 weeks Stratum corneum (SC) water content and Transepidermal water loss (TEWL) were monitored every week to measure any effect produced by these topical creams. The expected organoleptic stability of creams was achieved from 4 weeks in-vitro study period. Odor was disappeared with the passage of time due to volatilization of lemon oil. Both the Base and Formulation produced significant ($p \leq 0.05$) changes in TEWL with respect to time. SC water content was significantly ($p \leq 0.05$) increased by the Formulation while the Base has insignificant ($p > 0.05$) effects on SC water content. The newly formulated cream of Aloe Vera, applied is suitable for improvement and quantitative monitoring of skin hydration level (SC water content/ moisturizing effects) and reducing TEWL in people with dry skin.

Keywords—Aloe Vera; Skin; Stratum corneum (SC) water content and Transepidermal water loss (TEWL).

I. INTRODUCTION

AN emulsion is a system in which one fluid is dispersed in another with which it is immiscible. Macroscopic separation of the phases is prevented by the addition of a suitable surfactant. [1] A system that consists of oil droplets dispersed in an aqueous phase is called oil-in-water or O/W emulsion. And a system that consists of water droplets dispersed in an oil phase is called a water-in-oil or W/O emulsion. [2]

Emulsions are heavily used in the food industry, cosmetics and paints, the pharmaceutical industry, agricultural products and the petroleum industry. [3] There has been renewed

interest in the emulsion as a vehicle for delivering drugs to the body as it has been found to have several advantageous characteristics, frequently enhancing the bioavailability of the drug substance. In an emulsion the therapeutic properties and spreading ability of the constituents are increased. [4] Water-in-oil emulsions are employed more widely for the treatment of dry skin and emollient applications. [5] Additional value can be given to these formulations by including active ingredients with specific cosmetic effects. Particularly advantageous cosmetic emulsion preparations are obtained when antioxidants are used as active ingredients. [6]

Aloe Vera has been very extensively used in health foods, cosmetics, and traditional medicines. Various studies have revealed that substances isolated from Aloe possess many pharmaceutical activities, including anti-inflammatory, anti-oxidative, anti-aged, anti-cancer, and immunomodulatory, in which the mediation of ROS levels was involved. [7] Growing apprehensiveness about the safety of synthetic commercial antioxidants has prompted great efforts to screen active and stable antioxidants obtained from natural sources. Aloe Vera leaves, which are used traditionally for their therapeutic properties, have been studied for their potential antioxidant activity. In the previous research from our laboratory, the growth period played a vital role in the composition and antioxidant ability of ethanol extracts derived from A. Vera leaf. [8] Reports credit Aloe with antitumor, antidiabetic, and antityrosinase properties in addition to efficacy in healing wounds and burns, treatment of gastric ulcers. The action of aloe gel as a moisturizing agent is still popular concept. Many studies have reported the presence of polysaccharides, especially the acetylated mannans from Aloe Vera, as the main component of the file with minor amounts of various other components. [7] Aloesin inhibits human tyrosinase activity via a noncompetitive inhibition mechanism, whereas arbutin works via a competitive inhibition mechanism. [9] Aloe Vera contains important ingredients necessary for wound healing, such as vitamin C (ascorbic acid), amino acids, vitamin E, and zinc. Ascorbic acid enhances the synthesis of collagen and counterbalances collagen breakdown. Vitamin E is a fat-soluble vitamin found in Aloe that has proven antioxidant activity. It may help stabilize lysosomal enzymes needed to synthesize collagen and it prevents free radical damage (cross-linkage) that appears to be detrimental to normal wound healing. It was demonstrated in the authors' laboratory that zinc improved the tensile strength of wounds, thus improving healing. [10]

The aim of this study was to formulate and measure the effects of a W/O cream of Aloe Vera extract on skin moisture

Khan BA is with The Islamia university of Bahawalpur Pakistan 63100 (phone: 0333-9732578; fax: 062-2881215; e-mail: barki.gold@gmail.com).

Akhtar N is with The Islamia university of Bahawalpur Pakistan 63100 (e-mail: nakhtar567@hotmail.com).

Bashir S is with Department of Pharmacy, University of Sargodha (e-mail: sajidpharm@gmail.com).

Mahmood T, Khan HMS, Khan MS and Iqbal M are with The Islamia university of Bahawalpur Pakistan 63100

content and TEWL after its formulation and In-vitro characterization.

II. MATERIALS AND METHODS

i. Materials

Paraffin Oil (Merck KGaA Darmstadt, Germany)
 ABIL-EM 90 (Franken Chemicals, Gebinde)
 Aloe Vera Extract (Department of pharmacy, IUB, Pakistan)
 Distilled Water (Department of pharmacy, IUB, Pakistan)
 Centrifuge Machine (Hettich EBA 20, Germany)
 Cold Incubator (Sanyo MIR-153, Japan)
 Conductivity-Meter (WTW COND-197i, Germany)
 Corneometer MPA 5 (Courage + Khazaka, Germany)
 Tewameter MPA 5 (Courage + Khazaka, Germany)
 Digital Humidity Meter (TES Electronic Corp, Taiwan)
 Electrical Balance (Precisa BJ-210, Switzerland)
 Homogenizer (Euro-Star, IKA D 230, Germany)
 Hot Incubator (Sanyo MIR-162, Japan)
 pH-Meter (WTW pH-197i, Germany)
 Refrigerator (Dawlance, Pakistan)
 Rotary evaporator (Eyela, Co. Ltd. Japan)
 SPSS 12.0
 Water Bath (HH .S21 4, China)

ii. Methods

a . Plant Material

Aloe Vera whole leaves are used as the plant material. The identification of this plant was performed from Cholistan Institute of Desert studies at The Islamia University of Bahawalpur, Pakistan

b . Preparation of Hydro-Alcoholic Extracts

Hydro alcoholic mixture was prepared by mixing one liter each of analytical grade Ethanol and distilled water. One kg whole leaves, unpeeled, of Aloe Vera was cut into thin slices by knife and was put into a glass beaker. Hydro alcoholic mixture was added to it and macerated for 48 hours. Glass beaker was sealed with aluminium foil and kept in the laboratory. The beaker was shaken for 10 minutes after every 12 hours.

c . Filtration

Finally the macerated material of plant was filtered through several layers of muslin cloth for coarse filtration. The coarse filtrate was then filtered through a Whatman # 01 filter paper.

d . Evaporation

The filtrate so obtained was evaporated under reduced pressure at 40 °C in a Rotary vacuum evaporator. The process of evaporation was continued till concentrate reduced to one third of the starting volume. The reddish brown colored extract so obtained was collected in Stoppard glass tubes and stored in freezer at 0°C.

e . Preparation of emulsions

In this study, W/O emulsions were prepared by the addition of aqueous phase to the oily phase with continuous agitation

[11]. Oily phase consisted of paraffin oil (16%) and surfactant ABIL- EM 90 (4%) was heated up to 75°C±1°C. At the same time, aqueous phase consisting of water (q.s) was heated to the same temperature and then the Aloe Vera extract (3%) was added in it. After that, aqueous phase was added to the oil phase drop by drop. Stirring was continued at 2000 rpm by the mechanical mixer for about 15 minutes until complete aqueous phase was added, 2 to 3 drops of lemon oil were added during this stirring time to give good fragrance to the formulation. After the complete addition of the aqueous phase, the speed of the mixer was reduced to 1000 rpm for homogenization, for a period of 5 minutes, and then the speed of the mixer was further reduced to 500 rpm for 5 minutes for complete homogenization; until the emulsion cooled to room temperature.

Base was also prepared by the same above method and with same ingredients but without Aloe Vera extract.

f . Properties of Emulsions

Emulsion was analyzed to assure the formulation of desired emulsion.

g . Physical Analysis

Emulsion was analyzed organoleptically (color, thickness, look, feel) and physically (creaming and phase separation).

h . Types of Emulsions

Type of emulsion was analyzed by diluting the emulsion with oil and water separately.

i . pH Determination

pH value of freshly prepared emulsion and emulsions kept at different conditions were determined by a digital pH-Meter.

j . Electrical Conductivity

Values for electrical conductivity of freshly prepared emulsion and emulsions kept at different conditions were monitored by a digital Conductivity-Meter.

k . Centrifugation Tests

Centrifugal tests were performed for emulsions immediately after preparation. The centrifugal tests were repeated for emulsions after 24 hours, 7 days, 14 days, 21 days, and 28 days of preparation. The centrifugal tests were performed at 25°C and at 5000 rpm for 10 minutes by placing the 5g of sample in disposable stoppard centrifugal tubes.

l . Stability Tests

Stability tests were performed at different conditions for emulsions to note the effect of these conditions on the storage of emulsions. These tests were performed on samples kept at 8°C ±0.1°C (in refrigerator), 25°C ±0.10C (in incubator), 40°C ±0.1°C (in incubator) and 40°C ±0.1°C (in incubator) with 75% relative humidity (RH). Physical characteristic of simple emulsions, i.e. color,

m . Product Evaluation on Skin

11 volunteers were selected whose ages were in between 25 and 35 years. Male volunteers were included in this work. Prior to the tests, the volunteers were examined by a cosmetic expert for any serious skin disease or damage especially on cheeks and forearms. Before the study, every volunteer was provided with a volunteer protocol. This protocol stating the terms and conditions of the testing were signed by every volunteer individually. Volunteers were not informed about the contents of formulations. All the skin tests were done at 25°C and 40% relative humidity conditions. On the first day, patch test (Burchard test) was performed on the forearms of each volunteer to determine any possible reactions to the emulsions. On the second day, each volunteer was provided with two creams. One cream was base and the other one was formulation containing the active ingredients. Each cream was marked with "right" or "left" indicating application of that cream to the respective cheek. The creams were applied by the volunteers themselves as instructed for 56 days. Every individual was instructed to come on days 7, 14, 21, 28, 42 and 56 for the skin measurements.

n . Patch Test (Burchard Test)

On the first day of skin testing, patch tests were performed on the forearms of each volunteer. A 5cm X 4cm region was marked on both the forearms. Basic values for erythema and melanin were measured with the help of Mexameter. 1.0 g of base and formulation each were applied to the 5cm X 4cm marked regions separately on each forearm. The regions were covered with the surgical dressing after application. After 24 hours, dressings were removed and the measurements of erythema and melanin were repeated on both forearms.

o . Skin Moisture Content

With the help of a Corneometer, moisture of the skin was measured before the application of any cream and then on days 7, 14, 21, 28, 42 and 56.

p . Skin pH Values

pH value of the skin was measured with the help of a skin pH-Meter before application of creams and then on days 7, 14, 21, 28, 42 and 56.

q . Panel Test

Every individual was provided with a form prepared previously to test the sensory values of cream. This form consisted of seven parameters to be evaluated and every parameter was assigned 7 values from -5 to +5 indicating very bad to very good, respectively. This form was asked to be completed independently by each individual on day 56.

r . Trans Epidermal Water Loss (TEWL)

Net TEWL of the skin was measured by the aid of a Tewameter before application of creams and then on days 7, 14, 21, 28, 42 and 56.

s . Mathematical Analysis

The percentage changes for the individual values of different parameters, taken every week, of volunteers were calculated by the following formula;

$$\text{Percentage Change} = [(A - B) / B] * 100$$

Where;

A = Individual value of any parameter of 1st, 2nd, 3rd, 4th, 6th or 8th week

B = Zero hour value of that parameter

t .Statistical Analysis

The measured values obtained for different parameters (skin moisture, sebum, melanin, erythema, TEWL and pH) were analyzed using SPSS 12.0 on the PC computer (paired samples t-test for variation between the two preparations; two-way ANOVA for variation between different time intervals).

III. RESULTS AND DISCUSSION

i . Results

a . Stability of Emulsion

Stability of base and formulation kept at different storage conditions was studied and physical characteristics regarding the stability of base and formulation have been

b . Centrifugation Tests

Centrifugation tests for base and formulation kept at different storage conditions were performed and phase separation in samples kept at different storage conditions was observed for 28 days at different time intervals. No phase separation after centrifugation was found in any of the samples of base and formulation.

c . Electrical Conductivity Tests

Electrical conductivity values for base and formulation kept at different storage conditions for 28 days have been determined. No change in electrical conductivity was found in any sample of base and formulation. The value of electrical conductivity always remained zero.

d . pH Tests

pH values for base and formulation kept at different storage conditions up to 28 days have been determined and reported in Table 1.

TABLE I
pH VALUES FOR BASE AND FORMULATION KEPT AT 8°C, 25°C, 40°C AND 40°C + 75% RH

Time	8 °C		25 °C		40 °C		40°C + 75% RH	
	B	F	B	F	B	F	B	F
0 Hour	5.76	5.25	5.76	5.25	5.76	5.25	5.76	5.25
12	5.65	5.14	5.8	5.4	5.59	5.28	5.56	5.04
24	5.68	5.76	5.73	5.51	5.51	5.53	5.59	5.23
36	5.71	5.83	5.48	5.34	5.67	5.7	5.66	5.38
48	5.61	5.54	5.58	5.68	5.29	5.3	5.48	5.11
72	5.56	5.64	5.63	5.49	5.16	5.35	5.49	5.25
7	5.77	5.15	5.73	5.7	5.54	5.08	5.42	4.73
14	5.68	5.42	5.59	5.67	5.52	4.81	5.21	4.77
21	5.67	5.21	5.58	5.09	5.59	4.53	5.44	4.13
28	5.11	5.07	5.57	4.47	4.97	4.18	5.13	3.9

B= Base, F= Formulation
RH= Relative Humidity

e. Patch Test for Erythema and Melanin

Before the application of base and formulation to human volunteers, patch tests for melanin and erythema were performed. The values measured have been given in Table 2.

TABLE II
PERCENTAGE OF CHANGE IN MELANIN/ERYTHEMA IN CASE OF BASE AND FORMULATION AFTER 24 HOURS (PATCH TEST)

Volunteer no.	Percentage Change			
	Melanin		Erythema	
	B	F	B	F
1	-5.923	13.47	3.407	14.23
2	6.354	-8.025	-6.877	-8.939
3	3.000	-13.48	-5.581	-1.337
4	-7.507	-15.43	-0.605	9.623
5	2.482	-5.822	8.169	-13.79
6	-1.163	-26.32	0.211	14.44
7	-16.86	-7.456	7.773	7.531
8	-2.640	-8.362	-1.212	-8.687
9	-13.20	-32.67	-1.651	0.264
10	-8.959	-19.94	19.44	12.16
11	-2.857	-7.051	0.593	-0.833
Mean ±	-4.297±	-11.92±	2.151±	2.242±
SEM	2.129	3.656	2.234	3.016

B= Base, F= Formulation

f. Skin Moisture

The percentage of change in the measured skin moisture values after applications of base and formulation have been given in Figure 1.

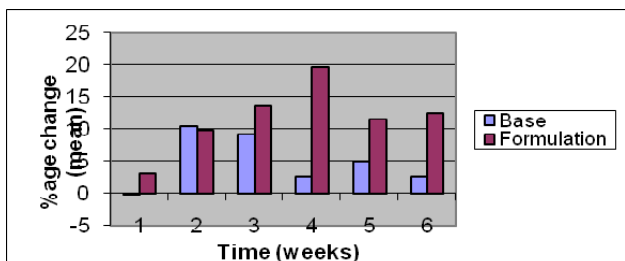


Fig. 1 Percentage of Change in skin Moisture Contents after application of Base and Formulation

Here 1= 1st week, 2= 2nd week, 3= 3rd week, 4= 4th week, 5= 6th week and 6= 8th week

g. pH of Skin

The percentage of change in the skin pH values after applications of base and formulation have been given in Figure 2.

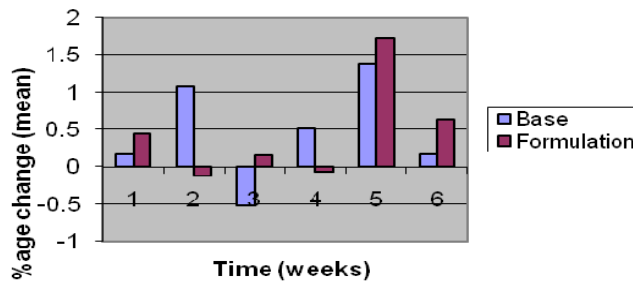


Fig. 2 Percentage of Change in skin pH after application of Base and Formulation

Here 1= 1st week, 2= 2nd week, 3= 3rd week, 4= 4th week, 5= 6th week and 6= 8th week

h. Panel Test

Sensory evaluation of the two creams by the volunteers has been presented in Figure 3.

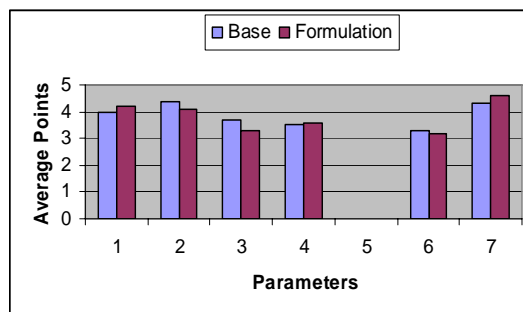


Fig. 3 Average Values For Panel Test

Here 1= Ease of application, 2= Spreadability, 3= Sense just after application, 4= Sense in long term, 5= Irritation, 6= Shine on skin, 7= Sense of softness.

i. Transepidermal water loss (TEWL)

The percentages of changes in transepidermal water loss (TEWL) values after applications of base and formulation have been given in Figure 4.

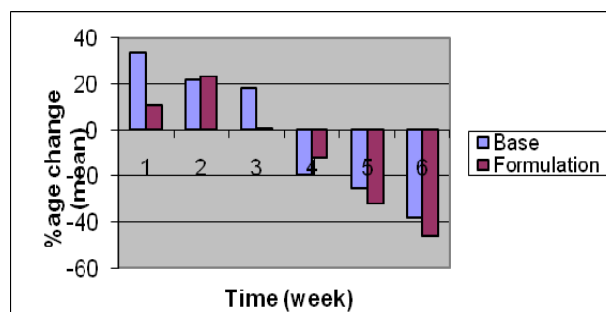


Fig. 4 Percentage of Change in TEWL after application of Base and Formulation

Here 1= 1st week, 2= 2nd week, 3= 3rd week, 4= 4th week, 5= 6th week and 6= 8th week

ii. Discussion

a . Stability Testing

“Oil in water” (O/W) or “water in oil” (W/O) emulsions, which represent the majority of cosmetic and pharmaceutical creams, evolve with time. They are thermodynamically unstable, usually splitting into two distinct phases. This instability could be manifested at different time rates and through a variety of physicochemical destabilizing processes, for example, creaming (or sedimentation), flocculation, coalescence or phase inversion. [13]

From a commercial viewpoint, it is important that new products be marketed as quickly as possible. However, such products should have a storage stability of several months at ambient temperature and under widely varying external influences. The final objective is to save time by predicting whether the emulsion is unstable or not before it breaks and separates into two layers visible by naked eye. [13] The readily apparent requirement in a well formulated emulsion is that the emulsion possesses adequate physical stability. [4]

The rate at which an emulsion breaks down will be strongly influenced by composition, environmental conditions (temperature, pH, etc.) and processing conditions. [14] It is argued that elevated temperature testing may successfully be applied to W/O emulsions provided that the phase inversion temperature is not crossed during the accelerated study. [15] The readily apparent requirement in a well formulated emulsion is that the emulsion possesses adequate physical stability. [16] A change in temperature has only an indirect effect, as a result altering the interfacial tension, adsorption of emulsifier and viscosity. However, significant changes in temperature cause changes in interfacial tension, viscosity, nature of the surfactants (hydrophilicity-Lipophilicity), and vapor pressure of the liquid phases and in the thermal agitation of the molecules. Thus, emulsions tend to be very sensitive to temperature changes. Emulsions are more stable when the temperature is near the point of minimum solubility of emulsifying agents. Emulsion stability decreases with increase in temperature. [17] Storage at various temperatures is a well known test method: the primary requirement of this accelerated test is that the temperature stress applied should speed up, but not alter the mechanism of deterioration operating under normal storage conditions. [13]

To obtain more information about the emulsion structure and its dependency on temperature and temperature cycling the rheological pre-stressed emulsion underwent temperature cycles from 5 to 40 °C within 24 h for a period of 1 month. These types of test are frequently used in pharmaceutical industry as accelerated tests in order to predict the long term stability in particular shelf-life of the investigated product. [18]

In this study, base and formulation were divided in to four samples separately and these samples were kept at different storage conditions i.e. at 8°C in refrigerator, at 25°C, 40°C and at 40°C + 75% RH (Relative Humidity) in stability chambers. These samples at different storage conditions were observed for a period of 28 days at definite time intervals. The samples were observed with respect to change in color, liquefaction and phase separation.

b . Color

The freshly prepared base was white and the formulation was creamy white in color. Regarding the base and the formulation there was no change in color up to the observation period of 28 days. This showed that the emulsions were stable at different storage conditions i.e. 8°C, 25°C, 40°C and 40°C + 75%RH throughout the period of analysis, i.e. 28 days.

No change in the color of base and formulation at the end of observation periods may be attributed to different factors contributing the emulsion stability. Such as the components of oil phase i.e. paraffin oil which is a colorless, transparent, tasteless, non-fluorescent liquid; and is mixture of hydrocarbons, [11] Abil-EM90 which is a clear, colorless and nontoxic liquid emulsifier. [12]

Aloe Vera's chemical composition also includes phenolic components with high antioxidant power, generally classified as cromones and anthraquinones. [19] Aloe-emodin is a natural active compound present in the leaves of Aloe Vera. Some studies have indicated that aloe-emodin has a number of biological properties, including antiviral, antimicrobial, and hepatoprotective activities. [20] It has been also reported to have antifungal properties. [21] Vitamin E is a naturally occurring tocopherol with antioxidant properties. [22] Thus it may protect the formulation components from microbial growth of these organisms which might produce such substances which are able to change the color of the formulation during the storage time.

c . Liquefaction

Viscosity is a useful process indicator of emulsion quality, as it is highly sensitive to changes in the emulsion due to variations in process and formulation parameters. [23] As soon as an emulsion has been prepared, time and temperature-dependent processes occur to effect its separation leading to the decreased viscosity which results in increased liquefaction. [4]

No liquefaction was observed in any of the sample of base and formulation kept at 8°C and 25°C during whole observation period of 28 days. Slight liquefaction was observed in the sample of base kept at 40°C on 28th day. Liquefaction was also observed in sample of base kept at 40°C + 75% RH from 21st day of observation but there was no further increase in liquefaction till the end of study period. On the other hand, a slight liquefaction was observed in formulation samples kept at 40°C + 75% RH on 28th day of observation period.

Creaming occurs when the density between the liquid (continuous phase) and the lipid (dispersed phase) is different. In creaming, there is buoyancy acting on the particles. Stoke's equation is usually used to define the velocity of the creaming rate. An understanding of the creaming rate helps to understand the stability of the emulsions. Creaming is related to the homogeneity of the particles, the size of the particles, and the viscosity of the system. The creaming rate will be reduced when the particles are homogeneous, small, the density difference between the particles is small, and the system is viscous. An emulsion with more small particles compared to large

particles of the same material, will result in higher viscosity which will reduce the creaming rate. [24]

d . Phase Separation

Creaming occurs when the density between the liquid (continuous phase) and the lipid (dispersed phase) is different. The separated phase can either cream or sediment. [24] Creaming is the upward movement of dispersed droplets relative to the continuous phase, while sedimentation, the reverse process, is the downward movement of particles. [4] The emulsions are not thermodynamically stable and droplets (water) merge with each other to produce big droplets and increase the coalescence rate. [17] Emulsions can deteriorate by creaming, flocculation, Ostwald ripening, or partial coalescence which leads to coalescence. [24] Coalescence is one of the possible mechanisms of destruction of emulsions, which occur when the energy of adhesion between two droplets is larger than the turbulent energy causing dispersion. [17]

The samples of base were stable at 8°C and 25°C but slight separation was observed at 40°C and 40°C+ 75% RH on 28th day of observation. While in the case of formulation, no phase separation was observed in any of the samples kept at 8°C, 25°C, 40°C and 40°C + 75% RH up to observation period of 28 days. This indicated that the formulation was relatively more stable than base at higher temperatures considering phase separation as a parameter of stability.

Slight phase separation in case of base at higher temperatures may be attributed to the movement of small number of surfactant molecules from interface to the surface [25] which is much easier when the emulsion has a lower viscosity. Depending on conditions, emulsions may be more stable at lower temperature due to increased phase viscosity. [14]

e . Centrifugation Test

Emulsion droplets are subjected to relative motion as a result of Brownian motions and high-intensity turbulence in emulsification systems and this leads to collision between droplets. Every collision does not necessarily result in re-coalescence; the intervening continuous liquid phase between droplets should drain, when the colliding droplets are close together. Between new droplet formation and its subsequent encounter with surrounding droplets, surfactants adsorb onto the created interface to prevent its re-coalescence. [26]

Centrifugation is based on the principle of using centrifugal force to separate two or more substances of different density, e.g., two liquids or a liquid and a solid and it is an extremely useful tool for evaluating and predicting the shelf life of emulsions. [4]

In this study centrifugation test was performed for the samples of the base and formulation kept at different storage conditions up to a period of 28 days at definite time intervals. No phase separation on centrifugation was seen in any of the samples kept at different storage conditions i.e. 8°C, 25°C, 40°C and 40°C+ 75% RH up to 28th day of observation. This indicated that the emulsions were stable at all the storage conditions for 28 days.

f . Electrical Conductivity

According to many authors (Delonca and Passet, 1973, Pearce and Kinsella, 1978, Kato et al., 1985 and Latreille and Paquin, 1990), conductimetry is often used to determine the nature of an emulsion and to control its stability during time. Actually, this method is sensitive to small changes in the emulsion's structure. Modifications of conductivity value allow the detection of creaming, sedimentation or phase inversion. [13] An emulsion in which continuous phase is aqueous can be expected to possess a much higher conductivity than an emulsion in which the continuous phase is oil. O/W emulsions will conduct, as water is the continuous phase, since oils are poor conductors, so W/O emulsions conduct poorly. [27]

In this study, conductivity test was performed for all the samples of base and the formulation kept at different storage conditions up to a period of 28 days at definite time intervals. No electrical conductivity was seen in any of the samples of base and formulation kept at different storage conditions i.e. 8°C, 25°C, 40°C and 40°C+ 75%RH up to 28th day of observation.

g . pH

Pharmaceutical stability studies include performances of drug in different forms and its pharmaceutical properties. Accelerated testing and pH-profile kinetics are the most important parts of chemical stability performances. [28] The pH is a significant parameter insofar as the effectiveness of the cream is concerned. The pH of human skin typically ranges from 4.5 to 6.0 [29] and 5.5 is considered to be average pH of the skin. Therefore, the formulations intended for application to skin should have pH closer to this range.

In this study, the pH of freshly prepared base and formulation was 5.76 and 5.25 respectively, which is within the range of skin pH. The pH values of the samples of base kept at different storage conditions i.e. 8°C, 25°C, 40°C and 40°C+ 75% RH was found to be increasing gradually in the 1st week and then it started to decline continuously till 28th day with some variations. At the end of study pH of the samples of base at 8°C, 25°C, 40°C and 40°C+ 75% RH was 5.11, 5.57, 4.97 and 5.13 respectively. Whereas pH of the samples of formulation kept at 8°C, 25°C, 40°C and 40°C + 75% RH showed gradual reduction in pH values with slight variations with time. The pH values of samples of formulation kept at 8°C, 25°C, 40°C and 40°C+ 75% RH were 5.07, 4.47, 4.18 and 3.9 at 28th day respectively.

By using two-way analysis of variance (ANOVA) technique at 5% level of significance, it was found that the change in pH of different samples of base and formulation were significant at different levels of time and temperature. The oxidation mechanism and the formation of oxidation products are enhanced by the presence of double bonds. [13]

h . Patch Test

In this study patch tests were performed on forearms of volunteers for 24 hours for both the base and formulation, to check the safety of the formulation and the base on human skin. It was found that erythema level was only slightly increased after the application of base and formulation by the end of 24 hours and mild skin irritation was produced by both the creams after 24 hours. But with paired sample t-test

it was evident that the effects of formulation and base were insignificant regarding the skin erythema.

It was concluded that the formulation and the base produced no skin irritation after performing patch test of 24 hours, so both creams can be used safely on human cheeks for in-vivo evaluation.

i . Skin Moisture Content

Formulation of this study contained Aloe Vera extract as an active ingredient which is rich in vitamins, especially vitamins B, C, E, β -carotene [30] and vitamin A. [31] Vitamin C is known to increase the collagen fibers in the dermis. With the increase in collagen, the hydration conditions in the dermis are improved. [32] The polysaccharide-rich composition of Aloe vera extracts (Aloe barbadensis Miller), often used in cosmetic formulations, may impart moisturizing properties to the product. [33]

In this study, it was found that there was a slight decrease in moisture value in 1st week after the application of the base and a very slight increase was observed at 2nd, 3rd, 4th, 6th and 8th week, while after the application of formulation there was the increase in moisture contents in whole of the study. With the help of ANOVA test it was found that the base showed insignificant change with respect to the basic values whereas the formulation showed significant variation throughout the study period of 8 weeks. With the help of paired sample t-test it was evident that significant differences in the moisture values were observed after application of base and formulation.

The significant increase in moisture after application of formulation may be because of Aloe vera extract that is a natural effective ingredient for improving skin hydration, possibly through a humectant mechanism. Consequently, it may be used in moisturizing cosmetic formulations and also as a complement in the treatment of dry skin. [33] Aloe Vera stimulates cell growth and as such enhances the restoration of damaged skin. [34]

j . Skin pH Values

The pH of human skin typically ranges from 4.5 to 6.0 [29] and 5.5 is considered to be average pH of the skin. Therefore, the formulations which are intended for application to skin should have pH closer to this range.

pH values of the skin of volunteers were measured at different time intervals before and after the application of base and formulation during the observation period of 8 weeks. It was observed that after the application of base pH values of the skin of volunteers were decreased in 3rd week while increased in 1st, 2nd and 4th, 6th and 8th week of study, whereas after the application of formulation pH values of the skin were decreased during 2nd and 4th week and slightly increased during 1st, 3rd, 6th and 8th week. With the help of ANOVA test, it was found that significant effects were shown after the application of base and insignificant effects were shown after the application of formulation. By applying paired sample t-test for base and formulation it was concluded that change in skin pH of volunteers was insignificant throughout the study period. It was also concluded that the formulation did not produce any alteration on the pH values of human skin. Therefore, the formulation may be considered to be safe with respect to the

effect on skin pH values. Insignificant changes in the pH of skin in case of formulation might be attributed to the presence of natural antioxidants [35] in its active ingredient which can prevent the oxidative degradation of the skin by scavenging the free radicals and maintains the natural integrity of human skin.

k . Panel Test

A questionnaire containing seven questions was prepared and the two copies of this form were given to each volunteer for sensory evaluation of the two creams. Average points were calculated from the points assigned by each volunteer for each question for both of the creams (base and formulation). Average points for the first question, i.e. ease of application of creams were found to be 4.00 and 4.20 for the base and formulation, respectively. This indicated that base and formulation can be easily applied on the skin. Average points regarding spread ability were 4.40 for base and 4.10 for formulation which meant that the formulation spread on skin better than the base. Average points for feel on application were 3.70 for the base and 3.30 for formulation. This indicated that base was felt well on the skin than formulation. Average points for the sense in long-term application of creams were 3.50 and 3.60 for the base and formulation respectively. This showed that formulation produced more pleasant feeling on application to skin than base. There was no irritation on the skin in both cases i.e. base and formulation, as these were assigned 0.00 point for irritation by all the volunteers. Shine on skin was 3.30 for the base and 3.20 for formulation. This was expected since the base and formulation contained same quantity of paraffin oil. Similarly, the formulation led to more softness of the skin than base as the average point was 4.30 for base and 4.60 for formulation.

It was found from paired sample t-test that there was an insignificant difference between the average points of sensitivity for base and formulation. It was concluded that there was no big variation between base and formulation regarding the sensory evaluation. Both of the creams behaved similarly from the sensory point of view.

l . Transepidermal water loss (TEWL)

Transepidermal water loss (TEWL) is the outward diffusion of water through skin. [36] TEWL is a measure of cutaneous barrier function and also reflects skin water content. [37] An increase in TEWL reflects an impairment of the water barrier. TEWL measurements allow parametric evaluation of the effect of barrier creams against irritants and characterization of skin functionality in clinical dermatitis and in irritant and allergic patch test reactions. TEWL measurements can be affected by the anatomical site, sweating, skin surface temperature, inter- and intra-individual variation, air convection, ambient air temperature, ambient air humidity, and instrument related variables. [36] In this study, it was found that there was increase in TEWL values after the application of base having the greatest value after 1st week then gradual reduction in loss and after formulation there was increase in TEWL after 1st, 2nd and 3rd week but decrease in the remaining period of study. With the help of ANOVA test, it was found that changes in TEWL produced by both formulation and base were

significant. By applying LSD test, it was found that in both the cases i.e. base and formulation the change in TEWL values became significant after 6th and 7th week of application. With the help of paired sample t-test it was found that there was significant variation in TEWL with respect to base and formulation in 2nd week of study while insignificant for other periods.

Emollient help prevent dryness while moisturizing and softening the skin. Applying an emollient provides a surface film of lipid and restores some of the some of the barrier function. This oily layer also helps to trap water under the stratum corneum, reducing epidermal water loss and making the skin softer and suppler. [38]

IV. CONCLUSION

As a conclusion of this study:

- A W/O base using paraffin oil and W/O formulation using extract of Aloe Vera and paraffin oil can be formulated.
- There was no change in color of base and formulation kept at all storage conditions for a study period of 4 weeks.
- A little phase separation was observed in the samples of base kept at 40°C and 40°C + 75% RH on 28th day but there was no phase separation in samples of formulation at all storage conditions for a period of 4 weeks.
- There was no liquefaction of base and formulation kept at 8°C and 25°C during whole observation period of 4 weeks but slight liquefaction was observed in the sample of base kept at 40°C on 28th day and in sample of base kept at 40°C + 75% RH from 21st day of observation. While only a slight liquefaction was observed in the samples of formulation kept at 40°C + 75% RH on 28th day of observation period.
- There was no phase separation in samples of base and formulation at all storage conditions for a period of 4 weeks in centrifugation test.
- Significant change was observed in pH values of base and that of formulation, at different storage conditions with time.
- Both base and formulation increased skin moisture contents at the end of study, so have the moisturizing effects.
- No pronounced change in pH of human skin was observed in case of Formulation.
- Both base and formulation decreases TEWL and this increase was significant statistically. Hence formulation increases the moistures contents by decreasing Trans epidermal water loss.

ACKNOWLEDGMENT

The authors thanks to Higher Education Commission of Pakistan for financial support and The Department of Pharmacy, The Islamia University of Bahawalpur for moral support.

REFERENCES

- [1] Imhof A and Pine D J. Stability of Nonaqueous Emulsions, *Journal of Colloid and Interface Science*, 1997:192, 368–374.
- [2] Armen K. Random centroid optimization of lutein-enriched oil in water emulsion at acidic pH, A Thesis Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College, 2003.
- [3] Timothy N H, Robert J P, George V F and Graeme J J. The role of particles in stabilizing foams and emulsions. *Advances in Colloid and Interface Science*, 2008: 137, 57–81.
- [4] Herbert A L, Martin M R and Gilbert S B. *Pharmaceutical Emulsions, Pharmaceutical Dosage Forms: Disperse System. Vol.1*, Marcel Dekkar, New York and Basel, 1988:199-240, 285-288.
- [5] Magdy I M. Optimization of Chlorphenesin Emulgel Formulation. *The AAPS Journal*, 2004, 6: 1-7.
- [6] Bleckmann A, Kropke R and Schneider G U. Preparations of the W/O emulsion type with increased water content, and comprising cationic polymers, United States Patent, 2006: 7138128.
- [7] Xiu Z, Hong W, Yuan S, Li N, Lan W, Bin L, Ping S and Yang L, Isolation, structure elucidation, antioxidative and immunomodulatory properties of two novel dihydrocoumarins from Aloe Vera. *Bioorganic & Medicinal Chemistry Letters*, 2006, 16: 949–953.
- [8] Qiuhui H, Yun H and Juan X. Free radical-scavenging activity of Aloe Vera (*Aloe barbadensis* Miller) extracts by supercritical carbon dioxide extraction. *Food Chemistry*, 2004, 91: 85-90.
- [9] Seongwon C. and Myung H. C. A review on the relationship between aloe Vera components and their biologic effects. *Seminars in Integrative Medicine*, 2003, 1: 53-62.
- [10] Robert H. D & Nicholas P. M, Aloe Vera and gibberellin anti-inflammatory activity in diabetes. *Journal of the American Podiatric Medical Association*, 1989, 79.
- [11] Henriette K. Paraffinum Liquidum, B.P. Liquid Paraffin. *The British Pharmaceutical Codex*, 1995:1911 <http://www.henriettesherbal.com/>.
- [12] Raymond C R, Paul J S, Paul J W. Dimethicone, Mineral Oil, Wax White; Wax Yellow. *Handbook of Pharmaceutical Excipients*, (4th Ed.), The PhP. London, Chicago, and the APhA. Washington, 2003: 213-214, 395-396, 687-690.
- [13] Masmoudi H, LE D. Y, Piccerelle P and Kister J, The evaluation of cosmetic and pharmaceutical emulsions, aging process using classical techniques and a new method: FTIR. *International Journal of Pharmaceutics*, 2005: 289,117-131.
- [14] Derick R. Fat crystals and emulsion stability, a review. *Food Research International*, 2000: 33, 3-14.
- [15] Bjerregaard S, Vermehren C, Soderberg I. and Frokjaer S. Accelerated Stability Testing of a Water-in-Oil Emulsion. *Journal of Dispersion Science and Technology*, 2001: 22, 23-31.
- [16] Herbert A L, Martin M R and Gilbert S B. *Emulsions and Microemulsions. Pharmaceutical Dosage Forms Disperse Systems.*, vol. 2, Marcel Dekker, INC, New York and Basel, 335-369, 1989.
- [17] Abdurahman H N and Rosli M Y. Stability investigation of Water-in-Crude oil emulsion. *Journal of Applied Sciences*, 2006: 6, 2895-2900.
- [18] Peter F, Andreas E, Erich J W AND Michael S. Predictive stress tests to study the influence of processing procedures on long term stability of supersaturated pharmaceutical o/w creams. *International Journal of Pharmaceutics*, 2007: 339, 189-196.
- [19] Antonio V, Elsa U, Roberto L and Margarita M, Hot-air drying characteristics of Aloe Vera (*Aloe barbadensis* Miller) and influence of temperature on kinetic parameters. *LWT*, 2007: 40, 1698–1707.
- [20] Sheng H C, Kai Y L, Chun C C, Chia L F and Chih P L, Aloe-emodin-induced apoptosis in human gastric carcinoma cells. *Food and Chemical Toxicology*, 2007: 45, 2296–2303.
- [21] Oana R C, Marcel P, Laurian V and Mircea T, Antifungal activity of Aloe Vera leaves. *Fitoterapia*, 2007: 78, 219–222.
- [22] Desai K N, Wei H, and Lamartiniere, The preventive and therapeutic potential of the squalene-containing compound, Roindex, on tumor promotion and regression, *Cancer Letters* 101, 93-96, 1996.
- [23] Ronald M B and Thomas F B. Use of viscosity as an in-line diagnostic for high internal phase emulsion generation, US Patent 5306734, 1994.
- [24] Anita G, Physical properties of emulsion stabilized by κ-casein before and after treatment with chymosin. A Thesis Submitted to the Office of Graduate Studies of Texas A&M University, 2005.
- [25] Onuki A., Viscosity of Emulsion with Surfactant. *Europhysics Letters*, 24, 1993: 151-156.
- [26] Seid M J, Elham A, Yinghe H and Bhesh B. Re-coalescence of emulsion droplets during high-energy emulsification. *Food Hydrocolloids*, 2007 [doi].

- [27] James S, Joseph T R and Orapin P R. Coarse dispersion. Remington, the science and practices of pharmacy, 20th edition, volume 1, Lipincott Williams and Wilkins, 2000:316-334.
- [28] Nor A S, Khalid A, Asmah R and Wan Z W. Vitamin C and Aloe Vera supplementation protects from chemical hepatocarcinogenesis in the rat Nutrition, 1998: 14, 846–852.
- [29] Issa T S, Philippe B, Raymond H, Michel H and Jacques D. Improved kinetic parameter estimation in pH-profile data treatment, International Journal of Pharmaceutics, 2000: 198, 39–49.
- [30] Jennifer L M, K L Campbell, Ibulaimu K, Philip F S and David J S. Evaluation of the effect of pH on in vitro growth of Malassezia pachydermatous. Can J Vet, 2003: 67, 1, 56–59.
- [31] Campestrini L H, Kuhnen S, Lemos P M M, Bach D B, Dias, P F and Maraschin M. Cloning protocol of aloe Vera as a study-case for “tailor-made” biotechnology to small farmers. J. Technol. Manag. Innov, 2006: Issue 5, 1, 76-79.
- [32] Padayatty S J and Levine M. New insights into the physiology and morphology of vitamin C. Journal of Canadian Medical Association, 2001: 164, 353-355.
- [33] Susi E D, Lorena R G And Patricia M B. Moisturizing effect of cosmetic formulations containing Aloe vera extract in different concentrations assessed by skin bioengineering techniques. Skin Research and Technology, 2006: 12, 241.
- [34] Agarry O O, Olaleye M T and Bello M C O, Comparative antimicrobial activities of aloe Vera gel and leaf. African Journal of Biotechnology, 2005: 4 (12), 1413-1414.
- [35] Tampa F L, Antioxidant-Rich Diets Improve Age-Related Declines in Mental Function of Rats. Science Daily, Research News, July 15th, 2002.
- [36] Jackie L and Howard M. The correlation between transepidermal water loss and percutaneous absorption: an overview. Journal of Controlled Release, 2005: 103, 291–299.
- [37] Ostlere L S, Taylor C, Baillod R and Wright S. Relationship between pruritus, transepidermal water loss, and biochemical markers of renal itch in haemodialysis patients. Nephrology Dialysis Transplant, 1994: 9, 1302-1304.
- [38] Odukoya, Jenkins, Ilori And Sofidiya. Control of oxidative stress with natural products: The potential of Nigerian traditional emollients. European journal of Scientific Research, 2005:10, 27-32.