Development of cetyl dimethicone based water-in-oil emulsion containing botanicals: Physical characteristics and stability

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Abstract: The aim of current research was to develop a water-in-oil emulsion containing grape seed extract for application in cosmeceuticals. Finally grinded dried grape seeds powder was extracted with hydro alcoholic mixture. Emulsions consisting of different concentrations of cetyl dimethicone (Abile EM90), the nonionic emulsifier, liquid paraffin as oily phase and water as aqueous phase were developed. Color, odor, pH, viscosity, liquefaction, phase separation, centrifugation and thermal stability of the formulated emulsions were observed at various storage temperatures i.e. 8±0.5°C, 25±0.5°C, 40±0.5°C and 40°C±0.5°C with 70% RH. The stable formulation consist of 16% mineral oil, 4% of ABIL EM 90®, 4% grape seeds extract, 1% rose oil and 75% distilled water. All the results derived from this study showed good stability over the three months study period which indicates w/o emulsion can be used as carrier of 4% grape seeds extract to enhance desired effects when applied topically.

Keywords: Grape seed extract, w/o Emulsion, stability, pH, electrical conductivity, and rheology.

INTRODUCTION

The choice of emulsifier is crucial not only for the formation of the emulsion but also for its long-term stability. Several classes of emulsion may be distinguished, namely oil-in-water (O/W), Water-in-oil and oil-in-oil (Boyd *et al.,* 1972).

Water-in-oil emulsions consists of the water phase, which is internal/dispersed phase, mixed with oil, which is continuous phase. This emulsion type is often more difficult to prepare and stabilize since it is most often based on totally non-emulsifiers. However recent advances in silicon chemistry and polymer chemistry have allowed preparation of excellent water-in-oil (W/O) emulsions (Saito & Shinoda, 1970). A real benefit of these vehicle emulsions is that they are readily spread on to the lipophilic skin and provide a film which is resistant to water wash off. This is how water resistant moisturizing cosmeceuticals are created (Waqas *et al.,* 2010). Since the emulsifiers used for these emulsions are lipophilic, meaning oil loving, they do not upset the lipid bilayer and thus will not damage the skin barrier. The W/O systems are formed, using nonionic emulsifier, where the oil is expected to be an external phase. A wide variety of emulsifiers are used in pharmacy and cosmetics to prepare cosmetic emulsions. Nevertheless, these emulsifiers are often responsible for allergies and irritations. Therefore it is very important to develop formulations of cosmetic emulsions with emulsifier that do not cause allergies and irritations (Rousseau, 2000).

A non-ionic emulsifier that is Polysiloxane polyalkyl polyether copolymer commonly known as ABIL EM 90® has been used as an emulsifying agent, which finely distribute the water droplets into the continuous oil phase. ABIL EM 90® is clear and viscous oil soluble liquid having the HLB value equal to 5. It is widely used as emollient and anti-foaming agent in the cosmeceutical emulsions. It has high compatibility with active ingredients and form very stable formulations (Ali *et al.,* 2012). Botanical extracts form the largest category of cosmeceutical additives found in market place today. They can be easily added to cleansers, moisturizers, astringents, treatment creams, colored cosmetics and face masks (Allemann & Baumann, 2009). Botanical extract for topical applications are considered safe by US food and drug administration, thus allowing the products to be marketed without obtaining status or being restricted by monographed ingredients. Today cosmetic formulators have access to plant material worldwide for incorporation into cosmeceuticals (Aburjai & Natsheh, 2003).

Grape seed (*Vitis vinifera*) is indigenous to southern Europe and Western Asia and is cultivated today in all temperature regions of the world. Grape seeds contain several active botanicals like flavonoids, polyphenols, anthocyanins, proanthocyanidins, procyanidines, and the stilbene derivative resveratrol (Ricardo *et al.,* 1991). Grape seed applied topically improved cutaneous photo protection to UVB, inhibits histamine synthesis and promotes wound healing. Grape seed protects

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deoxyribonucleic acid (DNA) against oxidation more effectively than vitamins C and E and stabilizes collagen and elastin by inhibiting matrix metalloproteinase. It treats chronic venous insufficiency (CVI) and postoperative edema in clinical studies. All of these functions of grape seed strongly suggest that it should improve photoaged skin and protect against further damage (Nichols & Katiyar, 2010).

The purpose of the current research is to develop and characterize a stable cosmetic water-in-oil emulsion loaded with 4% of grape seed extract so that, it can be used for its cosmetic effects on human skin

MATERIALS AND METHODS

Materials

Grape seeds were obtained from a local market of Bahawalpur, Pakistan and authenticated by the CIDS (Cholistan institute of desert plants studies), The Islamia University of Bahawalpur, Pakistan. For future reference, a voucher specimen (Voucher no. GS-LF-8-15-25) has been kept in the herbarium at CIDS, The Islamia University of Bahawalpur, Pakistan. Polysiloxane polyalkyl polyether copolymer (ABIL EM 90) was purchased from the Franken Chemicals Germany, n. Hexane & paraffin oil were purchased from Merk KGaA Darmstadt (Germany). Ethanol& acetone were taken from BDH England. Distilled water was prepared in the Cosmetics Laboratory, Department of Pharmacy, The Islamia University of Bahawalpur, Pakistan. *Apparatus* Centrifuge Machine (Hettich EBA 20, Germany), Cold Incubator (Sanyo MIR-153, Japan), Conductivity-Meter (WTW COND-197i, 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 (Orient, Pakistan), Rotary evaporator (Eyela, Co. Ltd. Japan). DV III Ultra programmable Rheometer (Brookfield USA).

METHODS

Preparation of botanical extract

Grape seeds were obtained from a local market in Bahawalpur, Pakistan. 200grams of raisins (Grape seeds/*Vitis venifera*) were well ground by a grinder and put into a glass beaker. One liter Hydroalcoholic mixture was made by mixing 70% methanol and 30% water. This mixture was then added to the glass beaker containing raisins. The beaker was sealed with aluminum foil and kept at room temperature for 72 hours. The beaker was then shaken for 10 minutes twice a day. After 72 hours the macerated material of plant was passed through 16 folds of muslin cloth for coarse filtration. The resulting filtrate was passed through a Whatman #01 filter paper. The filtrate obtained from the previous step was evaporated with the help of rotary evaporator under reduced pressure at temperature of 40°C. The evaporation was carried out till the concentrate remained one third of the original quantity. The process of extraction was completed at this step and dark brown extract was obtained, collected in a stoppered bottle and refrigerated.

Preparation of cosmetic emulsions

Oil phase comprised of paraffin oil and emulsifier (ABIL-EM 90) was heated up to 75^{o}C±1^{o}C**.** At the same time, aqueous phase comprising of water was also heated to the same temperature. After heating the grape seed extract was added to the heated water. After that, aqueous phase was added to the oil phase drop by drop with continuous stirring at 2000 rpm with the help of mechanical mixer for about 15 minutes until complete aqueous phase was added, 2 to 3 drops of rose oil as fragrant were added during this stirring time. As the aqueous phase completely added, the speed of the mixer was reduced to 1000 rpm for homogenization for a period of 5 minutes. After this the speed of the mixer was further reduced to 500 rpm for 5 minutes for complete homogenization; until the emulsion cooled to room temperature. 25 formulations of W/O emulsions were prepared with various concentration of emulsifier cetyl dimethicone (ABIL- EM90), liquid paraffin and distilled water as shown in table 1. All these formulations were noted with respect to color, phase separation and liquefaction for 25 days while keeping them at 25°C in incubator. The formulations A2, A16 and A21 were found stable at 25°C. Four samples of each of these three formulations were studied further for 21 days while keeping them at 8°C, 25°C, 40°C, and 40°C +75% RH. The sample A2 was found to be stable at all storage conditions and this sample was selected for further in-vitro study. It was observed for three months with respect to smell and color, type of emulsion, electrical conductivity, centrifugation, liquefaction and the pH.

Determination of type of emulsion

There are many methods available for the determination of emulsion type but the most commonly used are: Drop dilution method, Dye solubility method, Fluorescent test and Filter paper test. We adopted the drop dilution method for detection of emulsion type. A certain amount of emulsion was taken in test tube and diluted with certain amount of water. The emulsion was immiscible and did not dilute with water confirming this emulsion was w/o type.

STATISTICAL ANALYSIS

The specific data was evaluated using the statistical tool SPSS version 17.0 according to two-way ANOVA test defining a 5% level of significance. Standard deviation was calculated for every mean value.

Table 1: The composition of 25 formulations of w/o emulsions

Formulation code	Paraffin oil (%)	ABIL EM® 90 (%)	Grape seed extract (%)	Rose oil (%)	Distilled water (%)
AI	14	4	4	1	77
42	16	4	4	1	75
A3	18	4	4	1	73
A4	20	4	4	1	71
A5	22	4	4	1	69
A6	14	3.5	4	1	77.5
A7	16	3.5	4	1	75.5
A8	18	3.5	4	1	73.5
A9	20	3.5	4	1	71.5
A10	22	3.5	4	1	69.5
A11	14	3	4	1	78
A12	16	3	4	1	76
A13	18	3	4	1	74
A14	20	3	4	1	72
A15	22	3	4	1	70
A16	14	2.5	4	1	78.5
A17	16	2.5	4	1	76.5
A18	18	2.5	4	1	74.5
A19	20	2.5	4	1	72.5
A20	22	2.5	4	1	70.5
A21	14	2	4	1	79
A22	16	2	4	1	77
A23	18	2	4	1	75
A24	20	2	4	1	73
A25	22	2	4	1	71

RESULTS

Determination of type of emulsion

A certain amount of emulsion was taken in test tube and diluted with certain amount of water. The emulsion was immiscible and did not dilute with water confirming this emulsion was w/o type.

Color and smell

Physical characteristics and visualization regarding the stability of emulsion of the entire four samples kept at 8± 0.5°C, 25±0.5°C, 40±0.5°C and 40±0.5°C +75% RH were studied and presented in table 2. The freshly prepared emulsion samples were pinkish in color. The pinkish color of samples was due to the presence of grape seed extract. There was no change in the color of the emulsion samples up to the observation period of 90 days. This represents that emulsion formulation was stable at different storage conditions i.e. 8^oC, 25^oC, 40^oC and 40^oC + 75% RH throughout the period of observation, i.e. 90 days.

Liquefaction

All samples of emulsion formulation kept at 8^0C and 25^0C were stable for the period of 90 days and there was no liquefaction observed. Very little liquefaction was seen in the sample of base kept at 40^oC on 90^{th} day. However little liquefaction was seen in samples kept at 40^oC on 90^{th} day of observation period.

Phase separation

There was no phase separation in any of the samples of emulsion formulation kept at 8^0C, 25^0C, 40^0C and 40^0C+ 75% RH up to observation period of 90 days. Even at higher temperature no oily phase separation was monitored during the entire study duration. A number of stability factors like availing ABIL EM 90, which is a heat stable emulsifying agent.

Conductivity

For the determination of nature of an emulsion and to manage its stability with the passage of time the conductivity test is commonly used. The conductivity test allows the detection of sedimentation, creaming and phase inversion. The measurement of the conductivity of prepared emulsion was done with the help of Conductivity Meter (WTW COND-197i, Germany). The conductivity test of all the four samples kept at 8oC, 25^oC, 40^oC and 40^oC +75%RH was done immediately after preparation then repeated after, 24 hour and 7,14,21,28,60 and 90 days. Each sample observation was performed triplicately . Results showed no electrical conductivity was seen in any of the samples kept at

Table 2: Physical Characteristics of emulsion Kept at 8°C, 25°C, 40°C and 40°C +75% RH

		Fresh	After 12 hrs	After 24 hrs	After 36 hrs	After 48 hrs	After 7days	After 14days	After 21days	After 28days	After 60days	After 90days
Color	A	P	P	P	P	P	P	P	P	P	P	P
	B	P	P	P	P	P	P	P	P	p	P	P
	C	P	P	P	P	P	P	P	P	P	P	P
	D	P	P	P	P	P	P	P	P	P	P	P
Smell	A	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve
	B	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve
	C	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve
	D	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve
Liquefaction	A	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	B	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	C	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve
	D	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve
Phase Separation	A	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	B	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	C	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	D	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

P=Pinkish, -ve =No change, +ve=Slight change; A=At 8^{0}C; B=At 25^{0}C; C=At 40^{0}C; D=At 40^{0}C+75%RH (Relative Humidity).

Table 3: pH Values of emulsion Kept at 8± 0.5°C, 25± 0.5°C, 40± 0.5°C and 40± 0.5°C + 75% RH

Temperature Time	8±0.5°C	25±0.5°C	40±0.5°C	40±0.5°C+75%RH
0 Hour	5.16±0.02	5.16±0.02	5.16±0.03	5.16±0.01
12 Hours	5.13±0.03	4.80±0.01	4.74±0.01	4.92±0.02
24hours	4.16±0.01	4.13±0.02	4.20±0.04	4.64±0.05
36 hours	4.14±0.04	4.42±0.01	4.10±0.01	4.57±0.01
48 hours	4.09±0.02	4.15±0.03	4.07±0.02	4.29±0.02
7 Days	4.48±0.04	4.09±0.01	4.05±0.02	4.22±0.04
14 Days	4.40±0.02	4.48±0.04	4.44±0.08	4.10±0.01
21 Days	4.26±0.01	4.01±0.02	4.29±0.01	4.20±0.02
28 Days	4.33±0.05	4.16±0.01	4.10±0.02	4.15±0.01
60days	4.32±0.01	4.23±0.02	4.07±0.01	4.24±0.06
90 days	4.30±0.02	4.20±0.01	4.05±0.03	4.21±0.03

different storage conditions i.e. 8°C, 25°C, 40°C and 40°C+ 75% RH.

pH tests

Generally the pH range of human skin is from 4.05 to 6.0 and the average pH of human skin is considered to be 5.5, therefore dermatological products should have pH closer to 5.5. In the current findings the pH was measured for all the 4 samples kept at 8°C, 25°C, 40 ^{0}C and 40°C +75%RH by using digital pH-Meter (WTW pH-197i, Germany) immediately after preparation and then repeated after, 24 hour and 7,14,21,28,60 and 90 days. Each sample observation was performed triplicately. The pH of freshly prepared emulsion was 5.16, which is within the range of the pH of the skin. pH values of all four samples kept at 8°C, 25°C, 40°C and 40°C+75% RH showed a gradual decline with a little variation with time (table 3). By using two-way analysis of variance (ANOVA) technique at 5% level of significance, it was evident that the change in the pH of different samples was significant ($p<0.05$) at different intervals of time at different temperatures. pH at different storage conditions is the oxidation of paraffin oil, which produces aldehydes and organic acids. The decrease in pH is also might be due to the production of highly acidic byproduct from any of the acidic ingredients of the grape seed extract e.g. ellagic acid (Nassiri & Hosseinzadeh, 2009). As the pH determined at various intervals was with in the skin pH range, so the cosmetic emulsion can be used safely on human skin.

Viscosity

The determination of viscosity of emulsions was done with the help of DV-III ultra programmable rheometer. The viscosity of all four samples kept at different storage conditions (8°C, 25°C, 40°C and 40°C+75%RH) was evaluated for fresh preparations and then repeated after

Table 4: Viscosity Values (cP) of emulsion kept at 8± 0.5°C, 25± 0.5°C, 40± 0.5°C and40± 0.5°C + 75% RH for a period of 90 days

Temperature Time	8±0.5°C	25±0.5°C	40±0.5°C	40±0.5°C+75%RH
0 Hour	481.16±1.01	481.16±1.01	481.16±1.01	481.16±1.01
12 Hours	479.93±1.12	481.04±1.05	481.16±1.14	480.27±1.05
24hours	481.06±1.14	481.51±1.12	480.96±1.02	482.03±1.12
36 hours	481.21±1.12	482.26±1.05	481.09±1.09	480.57±1.09
48 hours	480.86±1.05	482.00±1.09	478.63±1.12	479.50±1.13
7 Days	481.06±1.14	481.99±1.13	474.21±1.32	481.26±1.01
14 Days	479.56±1.05	482.93±1.01	474.56±1.08	478.52±1.08
21 Days	479.70±1.03	483.08±1.05	472.26±1.07	481.95±1.09
28 Days	479.81±1.07	480.69±1.13	474.43±1.22	481.95±1.12
60days	480.16±1.12	480.92±1.09	473.06±1.32	481.43±1.07
90 days	480.16±1.05	481.05±1.03	473.81±1.14	480.05±1.09

different time intervals (12hour, 24hour, 48 hour, 7 days, 14 days, 21 days, 28 days, 60 days, 90 days). Each sample observation was performed triplicately (table 4). The viscosity of emulsion samples after 90 days kept at storage condition of 8°C, 25°C and 40°C +75%RH was not different from the viscosity of freshly prepared emulsion. Anyhow a slight decrease in viscosity of emulsion sample kept at 40°C was noticed.

DISCUSSION

The stability of the formulations throughout the observation period may be attributed to different contributing factors. Such as the ingredients of non-aqueous portion like paraffin oil, which is mixture of hydrocarbons and a transparent, non-fluorescent liquid and Abil-EM90 which is a clear, colorless and nontoxic liquid emulsifying agent (Khan *et al.,* 2010). Being the bioactive ingredient i.e. Grape seed extract contain polyphenols (Mandic *et al.,* 2008). Polyphenols contain established antimicrobial activity against a vast majority of bacteria such as *Shigella flexneri, Staphyllococcus aureus.* Oxidized polyphenols also have inhibitory activity against bacterial growth. The mechanism of polyphenol toxicity against microbes may be the inhibition of hydrolytic enzymes. This may play a role in protecting the formulation from degradation by those organisms that produce such substances, which bring about changes in the color of the formulation during storage (Silván *et al.,* 2013).

In W/O emulsion, the creaming takes place due to the sedimentation of water droplets and forms the lower layer. According to the Stokes law, the rate of sedimentation is inversely proportional to the viscosity of the continuous phase. Due to this factor on increasing creaming, the viscosity of the formulation and the base gradually decreases at increased temperature, which leads to liquefaction (Onuki, 1993).

No electrical conductivity is an indication of non-conductive nature of oily phase in water-in-oil emulsion, where the oil is in continues phase .No conductivity is also attributed to the use of ABIL EM90, a non-ionic emulsifying agent. Anyhow, at the end of study duration a slight conductivity (Average 0.005μS/cm) was observed in the sample kept at 40°C which may occurred due to the production of acidic species, slight liquefaction and appearance of aqueous phase (Matousek *et al.,* 2003).

The possible reason for the reduction in the pH at different storage conditions is the oxidation of paraffin oil, which produces aldehydes and organic acids. The decrease in pH is also might be due to the production of highly acidic byproduct from any of the acidic ingredients of the grape seed extract e.g. ellagic acid (Nassiri & Hosseinzadeh, 2009). As the pH determined at various intervals was with in the skin pH range, so the cosmetic emulsion can be used safely on human skin.

It was observed from the different study that when temperature was increased, the flow of molecules through interface is also increased. The flow of molecule correlated with viscosities. The viscosity is very sensitive to the temperature hence; the increment in temperature caused reduction of emulsion viscosity (Farah *et al.,* 2005).

CONCLUSION

The w/o cream developed as a carrier for 4% grape seeds extracts, was found to be stable. From these findings it is possible to use the cream of grape seeds for skin care products, such as an anti-aging w/o emulsified system. Such a cream could provide a satisfactory effect if applied to the skin frequently.

It can also be concluded that the cosmetic w/o emulsion of grape seeds imparting a unique emulsion delivery system for skin rejuvenation. Further *in vivo* study should be performed on the skin of human volunteers for the evaluation of the cosmetic emulsion for commercial market by using non-invasive bioengineering techniques.

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