



Stability Study of a Cosmetic Emulsion Loaded with *Tamarindus indica* Seeds Extract

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SUMMARY. The aim of this study was to explain the physical stability (color, creaming, liquefaction, pH, conductivity, and centrifugation) of a new formulation of a cosmetic emulsion loaded with *Tamarindus indica* seeds extract. The formulation consists of polysiloxanepolyalkyl polyether copolymer (ABIL EM 90) which is a nonionic surfactant used as emulsifier and is characterized and monitored for various physico-chemical aspects. Twenty five formulations of W/O emulsions having different concentrations of oil and aqueous phases were prepared and analyzed for various *in vitro* parameters using suitable instruments. Physical stability was assessed by storing the formulations at 8°C, 25°C, 40°C, and 40°C with 75% RH (relative humidity) for a period of three months. The stable formulation consists of 4% *T. indica* seeds extract, 14% paraffin oil, 2.5% ABIL EM 90, 1% lemon oil and 78.5% distilled water. All the results derived from this study showed good stability over 12 week study period which indicate W/O emulsion can be used as carrier for 4% *T. indica* seeds extract to enhance desired effects.

RESUMEN. El objetivo de este estudio fue explicar la estabilidad física (color, formación de crema, licuefacción, pH, conductividad y centrifugación) de una nueva formulación de una emulsión cosmética conteniendo extracto de semillas de *Tamarindus indica*. La formulación consiste en un copolímero de poliéter polisiloxanopolialquilo (ABIL EM 90) y un tensioactivo no iónico usado como emulsionante, que se ha caracterizado y controlado por medio de diversos aspectos fisicoquímicos. Se prepararon y analizaron veinticinco formulaciones W/O que tienen diferentes concentraciones de aceite y fase acuosa mediante diversos parámetros *in vitro* utilizando instrumentos adecuados. La estabilidad física se evaluó mediante el almacenamiento de las formulaciones a 8 °C, 25 °C y 40 °C y 40 °C con 75% HR (humedad relativa) durante un período de tres meses. La formulación estable contiene 4 % de extracto de semillas de *T. indica*, 14 % de aceite de parafina, 2,5 % de ABIL EM 90, 1 % de aceite de limón y 78,5 % de agua destilada. Todos los resultados derivados de este estudio mostraron una buena estabilidad durante 12 semanas, indicando que la emulsión W/O puede ser utilizada como portador para que el extracto al 4 % de semillas de *T. indica* pueda lograr los efectos deseados.

INTRODUCTION

Emulsions are widely used in the pharmaceutical industry, agriculture, food products, paints and cosmetics¹. There has been revived pursuit in the emulsion as a medium for administering drugs to the body. Emulsions have several distinctive tendencies, mainly intensifying the bioavailability of the active pharmaceutical ingredients. In emulsions there is enhanced therapeutic outcome due to enhanced spreading ability of the constituents². W/O emulsions are frequently utilized for the skin dryness issues

and for emollient purposes³. Moreover, emulsions having botanical extracts show specific dermocosmetic effects when these extracts having antioxidant properties⁴. The W/O systems are formed using nonionic surfactants only in a temperature range above the HLB temperature, where the oil is expected to be a continuous 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 formu-

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lations of cosmetic emulsions with emulsifier that do not cause allergies and irritations ⁵. A non-ionic surfactant that is polysiloxanepolyalkylpolyether 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 dermocosmetic emulsions. It has high compatibility with active ingredients and form very stable formulations ⁶.

Nowadays, various plant extracts having specific properties are being used in the dermocosmetic preparations to enhance beauty and attractiveness ⁷. The use of plant extracts in dermocosmetics has minimum adverse effects to the skin and it largely depends on the nature of the constituents and the way they are formulated ⁸.

Tamarindus indica L. belongs to the Leguminosae family (Caesalpinioideae). The fruit has an elongated pod, 5 to 15 cm long, with dark brown bark, woody and brittle seeds in numbers from 3 to 8, surrounded by a brown pulp containing sugar, tartaric, acetic and citric acids. It is one of the most important fruits used as spice and food source in Africa ⁹. Its sweet and sour pulp and fibrous texture is used for preparing sweets, ice cream, liquors, soft drinks and concentrated juices. Practically all parts of the plant are used in folk medicine and it has numerous therapeutic applications in humans including its usage as digestive, tranquilizer, laxative and expectorant ¹⁰. *T. indica* seeds are important sources of antioxidant activity as 2-hydroxy-3',4'-dihydroxyacetophenone, metdihydroxybenzoate, 3,4-dihydroxyphenylacetate and (-)-epicatechin, in addition to oligomeric proanthocyanidins (OPC) ¹¹.

The shelf life is the time period for which a product may be stored before it becomes unfit for use because of chemical decomposition and/or physical deterioration ⁸. In the present study we designed a cosmetic emulsion loaded with botanical extract and examined its stability evaluation over a three month study period and investigated the changes on physical stability (color, creaming, liquefaction, pH, conductivity, centrifugation). The purpose of the current study is to develop and characterize a stable cosmetic emulsion loaded with 4% of *T. indica* seeds extract so that, it could be explored for its cosmetic effects in the carrier system.

MATERIALS AND METHODS

Materials

Tamarindus indica 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 (no. TI-SD-6-15-87) has been kept in the herbarium at CIDS, The Islamia University of Bahawalpur, Pakistan. Polysiloxanepolyalkyl polyether copolymer (ABIL EM 90 or cetyl PEG/PPG-10/1 dimethicone, was purchased from the Franken Chemicals Germany. n-Hexane and paraffin oil were purchased from MerckGAA Darmstadt (Germany). Ethanol and acetone were taken from BDH England. Distilled water was prepared in the Cosmetics Laboratory, Department of Pharmacy, The Islamia University of Bahawalpur, Pakistan.

Preparation of botanical extract and emulsions

Tamarindus indica seeds were obtained from a local market in Bahawalpur, Pakistan. They were extracted with hexane-ethanol-acetone (50:25:25). Oil phase comprised of paraffin oil and surfactant (ABIL-EM 90) was heated up to 75 ± 1 °C. At the same time, aqueous phase comprising of water was also heated to the same temperature. After heating the *T. indica* seeds 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 min until complete aqueous phase was added, 2 to 3 drops of lemon 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 min. After this the speed of the mixer was further reduced to 500 rpm for 5 min for complete homogenization, until the emulsion cooled to room temperature (Fig. 1). Base was also prepared by the same method and with same ingredients but without the *Tamarindus indica* seeds extract.

The 25 formulations of W/O emulsions were prepared with various concentrations of emulsifier, polysiloxanepolyalkyl polyether copolymer (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 (Sanyo MIR-162,

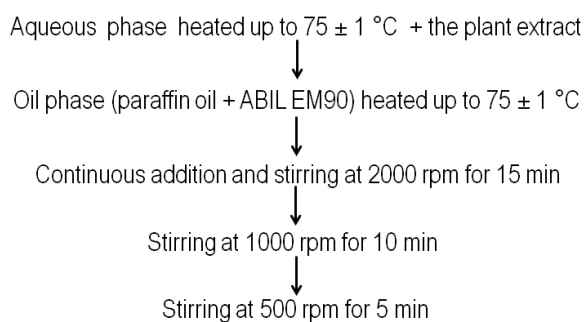


Figure 1. Preparation of cosmetic emulsions.

Japan). The formulations F7, F16 and F21 were found stable at $25 \text{ }^\circ\text{C}$. Four samples of each of these three formulations were studied further for 21 days while keeping them at $8 \text{ }^\circ\text{C}$, $25 \text{ }^\circ\text{C}$, $40 \text{ }^\circ\text{C}$, and $40 \text{ }^\circ\text{C} + 75\% \text{ RH}$. The sample F16 was found to be stable at all storage conditions as shown in Table 2 and this sample was selected

for further *in vitro* study. Both formulation and base were evaluated for three months with respect to smell and color, type of emulsion and electrical conductivity, centrifugation and liquefaction and pH.

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.

RESULTS AND DISCUSSION

Emulsions were examined organoleptically (odor and color) and physically (phase separation and creaming/sedimentation). These evaluations were carried out for a period of three months at different storage conditions ($8 \text{ }^\circ\text{C}$, $25 \text{ }^\circ\text{C}$, $40 \text{ }^\circ\text{C}$, and $40 \text{ }^\circ\text{C} + 75\% \text{ RH}$) and at specific

| Formulation code | Paraffin oil (%) | ABIL EM 90 (%) | Plant extract (%) | Lemon oil (%) | Distilled water (%) |
|------------------|------------------|----------------|-------------------|---------------|---------------------|
| F1 | 14 | 4 | 4 | 1 | 77 |
| F2 | 16 | 4 | 4 | 1 | 75 |
| F3 | 18 | 4 | 4 | 1 | 73 |
| F4 | 20 | 4 | 4 | 1 | 71 |
| F5 | 22 | 4 | 4 | 1 | 69 |
| F6 | 14 | 3.5 | 4 | 1 | 77.5 |
| F7 | 16 | 3.5 | 4 | 1 | 75.5 |
| F8 | 18 | 3.5 | 4 | 1 | 73.5 |
| F9 | 20 | 3.5 | 4 | 1 | 71.5 |
| F10 | 22 | 3.5 | 4 | 1 | 69.5 |
| F11 | 14 | 3 | 4 | 1 | 78 |
| F12 | 16 | 3 | 4 | 1 | 76 |
| F13 | 18 | 3 | 4 | 1 | 74 |
| F14 | 20 | 3 | 4 | 1 | 72 |
| F15 | 22 | 3 | 4 | 1 | 70 |
| F16 | 14 | 2.5 | 4 | 1 | 78.5 |
| F17 | 16 | 2.5 | 4 | 1 | 76.5 |
| F18 | 18 | 2.5 | 4 | 1 | 74.5 |
| F19 | 20 | 2.5 | 4 | 1 | 72.5 |
| F20 | 22 | 2.5 | 4 | 1 | 70.5 |
| F21 | 14 | 2 | 4 | 1 | 79 |
| F22 | 16 | 2 | 4 | 1 | 77 |
| F23 | 18 | 2 | 4 | 1 | 75 |
| F24 | 20 | 2 | 4 | 1 | 73 |
| F25 | 22 | 2 | 4 | 1 | 71 |

Table 1. Composition of 25 formulations of W/O emulsions.

| Cosmetic emulsion | Paraffin oil (%) | ABIL EM 90 (%) | Plant extract (%) | Fragrance (lemon oil, %) | Distilled water (%) |
|--------------------------|------------------|----------------|-------------------|--------------------------|---------------------|
| Base | 14 | 2.5 | Nil | 1 | 82.5 |
| Stable formulation (F16) | 14 | 2.5 | 4 | 1 | 78.5 |

Table 2. Composition of base and stable formulation.

| Physical Characteristics | Fresh | | After 12 h | | After 24 h | | After 48 h | | After 7 d | | After 14 d | | After 21 d | | After 28 d | | After 60 d | | After 90 d | | |
|--------------------------|-------|---|------------|---|------------|---|------------|---|-----------|---|------------|---|------------|---|------------|---|------------|---|------------|---|---|
| | B | F | B | F | B | F | B | F | B | F | B | F | B | F | B | F | B | F | B | F | |
| Color | A | W | P | w | P | w | P | w | P | W | P | W | P | w | P | w | P | w | P | w | P |
| | B | W | P | w | P | w | P | w | P | W | P | W | P | w | P | w | P | w | P | w | P |
| | C | W | P | w | P | w | P | w | P | W | P | W | P | w | P | w | P | w | P | w | P |
| | D | W | P | w | P | w | P | w | P | W | P | w | P | w | P | w | P | w | P | w | P |
| Liquefaction | A | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | B | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | C | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| | D | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | + | + |
| Phase Separation | A | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | B | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | C | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |
| | D | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - |

Table 3. Physical Characteristics of Base (B) and Formulation (F) Kept at 8 °C, 25 °C, 40 °C and 40 °C +75% RH. W: white; P: pinkish; -: no change; +: little change; A: at 8 °C; B: at 25 °C; C = at 40 °C; D: at 40 °C + 75% RH.

time intervals. Organoleptic and physical properties concerning the stability of formulation and the base have been presented in Table 3.

Color

The freshly prepared base was white and formulation was pinkish in color. The pinkish color of formulation was due to the presence of *Tamarindus indica* seeds extract which contains carotenoids¹². There was no change in the color of both the base and the formulation up to the observation period of 90 days. This showed that both of the formulations 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.* 90 days. The stability of the formulations throughout the observation period may be attributed to different contributing factors. The ingredients of non-aqueous portion are paraffin oil, which is a mixture of hydrocarbons and a transparent, non-fluorescent liquid¹³ and ABIL-EM90, which is a clear, colorless and non-toxic liquid emulsifying agent⁶. The active ingredient *i.e.* *T. indica* seeds extract, contain polyphenols¹¹, which have established antimicrobial activity against majority of bacteria such as *Shigella flexneri* and *Staphylococcus aureus*¹⁴. Oxidized polyphenols also have inhibitory activity against bacterial growth. The mechanism of polyphenol toxicity against microbes may be the inhibition of hydrolytic enzymes¹⁴. 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 the storage.

Liquefaction

The viscosity of emulsions is a key factor in their flow characteristics¹⁵. Viscosity is a very practicable tool to check the quality and stability of the emulsion¹⁶. As time elapses the prepared emulsions are prone to temperature and time dependent degradative processes which leads to decrease in viscosity and increase in liquefaction². All samples of formulation and base kept at 8 and 25 °C were stable for the period of 90 days and no liquefaction was seen in them. Very little liquefaction was seen in the sample of base kept at 40 °C on 90th day. Likewise a little liquefaction was also seen in formulation samples kept at 40 °C on 90th day of observation period. 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, viscosity of the formulation and the base gradually decreases at increased temperature which leads to liquefaction¹⁷.

Phase separation

In emulsions, the difference in the densities of the water and oil phase under the influence of gravity causes creaming which results in phase separation¹⁸. The separated phase may be the sediment or cream. Sedimentation is the

downward movement of the dispersed phase while the creaming is the upward movement of the dispersed globules². Moreover, the emulsions are thermodynamically unstable systems and droplets of the dispersed phase combine with each other to form bigger droplets and cause an increase in the coalescence rate. Coalescence is one of the underlying causes of breaking of emulsions. Coalescence occurs when the turbulent energy causing dispersion of the globules is lower than the adhesive energy of the globules¹⁹. The samples of base which were kept at 8 and 25 °C show stability but slight separation was shown by the samples those were kept at 40 °C and 40 °C + 75% RH on 90th day of observation, while in case of samples of formulation, there was no phase separation in any of the samples kept at 8 °C, 25 °C, 40 °C, and 40 °C + 75% RH up to observation period of 90 days. If phase separation is considered as a parameter of stability the formulation shows better stability than the base at higher temperatures. In the case of base, little phase separation at higher temperatures may be due to the movement of small number of molecules of the emulsifying agent from interface to the surface. This phenomenon is much easier at the low viscosity of the emulsion²⁰. In some conditions emulsions show higher stability at low temperatures because of increased phase viscosity¹⁸.

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 type²¹. 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.

Centrifugation test

The process of centrifugation is based on the principle of separating the two or more substances having different densities using the centrifugal force, for example two liquids or a liquid and a solid. This is a very useful tool for evaluation and prediction of the shelf life of emulsions². In the present study centrifugation test was employed to check the phase separation in the samples of base and the formulation kept at different temperatures up to a period of

90 days at definite time intervals. On centrifugation, there was no phase separation observed 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 90th day of observation. This showed that the emulsions were stable at all the storage conditions for 90 days. During emulsion formulation, the proper homogenization speed is very critical as it prevents the base and the formulation breakage during stress testing¹⁹.

Electrical conductivity

An emulsion which contains water as continuous phase is expected to possess a much higher conductivity than an emulsion in which the continuous phase is oil. So O/W emulsions will conduct electricity but W/O emulsions conduct poorly since oils are poor conductors²². The conductivity differences in the emulsions emerge when there is creaming and the proportion of water increases in the lower part of emulsion while the proportion of oil increases in the upper part. Conductivity test is also used to distinguish between the two emulsion types. In the present study, conductivity test was performed for a period of 90 days at definite time intervals for all the samples of formulation and the base kept at different storage conditions. Results showed no electrical conductivity in any of the sample of formulation and the base kept at different storage conditions *i.e.* 8 °C, 25 °C, 40 °C, and 40 °C + 75% RH.

Viscosity

The flow property of emulsions is highly dependent on its viscosity. The determination of viscosity was done with the help of DV-III ultra-programmable rheometer (Brookfield, USA). The viscosity of all four samples of base and formulation 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 different time intervals (12 h, 24 h, 48 h, 7 d, 14 d, 21 d, 28 d, 60 d, and 90 d). Viscosities were also found to decrease in both base and formulations kept at different storage conditions, especially at 40 °C (Table 4, Figs. 2A and 2B). 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 viscosity²³.

pH tests

Human skin typically has a pH ranges from 4.5 to 6.0 while 5.5 is considered to be the average pH of the human skin ²⁴. Therefore, the formulations should have pH closer to this range if it is intended for application to the skin. In the present study the pH of the base and formula-

| Time (days) | Temperature | Mean viscosity of base (cP) | Mean viscosity of formulation (cP) |
|-------------|----------------|-----------------------------|------------------------------------|
| 0 | 8 °C | 195.32 ± 1.05 | 152.56 ± 1.14 |
| 15 | | 187.22 ± 1.12 | 149.89 ± 1.02 |
| 30 | | 185.22 ± 1.07 | 149.43 ± 1.09 |
| 45 | | 180.22 ± 1.03 | 147.23 ± 1.12 |
| 60 | | 172.59 ± 1.05 | 145.29 ± 1.32 |
| 75 | | 169.74 ± 1.14 | 144.52 ± 1.08 |
| 90 | | 165.53 ± 1.05 | 143.38 ± 1.07 |
| 0 | 25 °C | 195.32 ± 1.05 | 152.56 ± 1.14 |
| 15 | | 184.68 ± 1.12 | 145.29 ± 1.32 |
| 30 | | 182.32 ± 1.14 | 145.11 ± 1.22 |
| 45 | | 168.91 ± 1.12 | 143.38 ± 1.07 |
| 60 | | 165.22 ± 1.01 | 142.55 ± 1.09 |
| 75 | | 158.32 ± 1.05 | 140.46 ± 1.12 |
| 90 | | 155.30 ± 1.13 | 140.55 ± 1.07 |
| 0 | 40 °C | 195.32 ± 1.05 | 152.56 ± 1.09 |
| 15 | | 160.62 ± 1.08 | 145.52 ± 1.08 |
| 30 | | 151.22 ± 1.01 | 143.55 ± 1.09 |
| 45 | | 149.39 ± 1.22 | 141.46 ± 1.12 |
| 60 | | 145.32 ± 1.13 | 139.48 ± 1.12 |
| 75 | | 142.30 ± 1.12 | 135.78 ± 1.09 |
| 90 | | 139.12 ± 1.12 | 125.26 ± 1.07 |
| 0 | 40 °C + 75% RH | 195.33 ± 1.05 | 150.51 ± 1.09 |
| 15 | | 146.35 ± 1.01 | 142.52 ± 1.09 |
| 30 | | 144.30 ± 1.13 | 143.55 ± 1.07 |
| 45 | | 132.22 ± 1.09 | 135.46 ± 1.03 |
| 60 | | 135.78 ± 1.05 | 134.11 ± 1.09 |
| 75 | | 135.31 ± 1.12 | 133.52 ± 1.097 |
| 90 | | 126.25 ± 1.05 | 131.30 ± 1.13 |

Table 4. Viscosities of base and formulation during storage for 3 months.

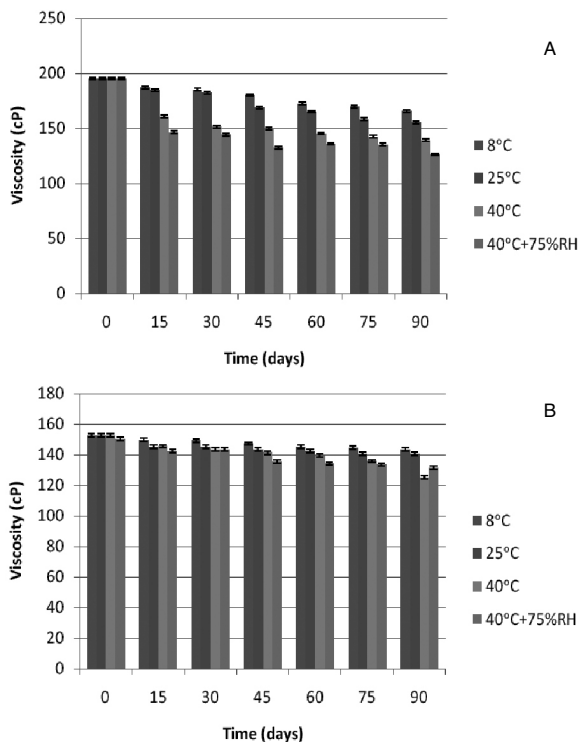


Figure 2. Viscosity of the base (A) and the formulation (B) at different times and temperatures.

tion was measured for all the 4 samples kept at 8 °C, 25 °C, 40 °C and 40 °C + 75% RH by using digital pH-meter (WTWpH-197i, Germany) immediately after preparation and then repeated after, 24 h and 7, 14, 21, 28, 60 and 90 d. The pH of freshly prepared base was 5.36 and of formulation was 5.88, which is within the range of the pH of the skin. 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 showed a gradual increase in pH in the 1st week and then it showed a continuous decline till 90th day with some variations. At the end of study pH of the samples of base at 8 °C, 25 °C,

| Time | Temperature | | | |
|------|-------------|-------------|-------------|----------------|
| | 8 °C | 25 °C | 40 °C | 40 °C + 75% RH |
| 0 h | 5.36 ± 0.02 | 5.36 ± 0.02 | 5.36 ± 0.03 | 5.36 ± 0.01 |
| 12 h | 5.25 ± 0.03 | 5.51 ± 0.01 | 5.39 ± 0.01 | 5.15 ± 0.02 |
| 24 h | 5.87 ± 0.01 | 5.62 ± 0.02 | 5.64 ± 0.04 | 5.34 ± 0.05 |
| 48 h | 5.94 ± 0.02 | 5.45 ± 0.03 | 5.81 ± 0.02 | 5.49 ± 0.02 |
| 7 d | 5.65 ± 0.04 | 5.79 ± 0.01 | 5.41 ± 0.02 | 5.22 ± 0.04 |
| 14 d | 5.75 ± 0.02 | 5.60 ± 0.04 | 5.46 ± 0.08 | 5.36 ± 0.01 |
| 21 d | 5.26 ± 0.01 | 5.81 ± 0.02 | 5.19 ± 0.01 | 4.84 ± 0.02 |
| 28 d | 5.53 ± 0.05 | 5.78 ± 0.01 | 4.92 ± 0.02 | 4.88 ± 0.01 |
| 60 d | 5.32 ± 0.01 | 5.20 ± 0.02 | 4.64 ± 0.01 | 4.24 ± 0.06 |
| 90 d | 5.18 ± 0.02 | 4.58 ± 0.01 | 4.29 ± 0.03 | 4.01 ± 0.03 |

Table 5. pH values for base kept at 8 °C, 25 °C, 40 °C and 40 °C + 75% RH.

| Time | Temperature | | | |
|------|-------------|-------------|-------------|----------------|
| | 8 °C | 25 °C | 40 °C | 40 °C + 75% RH |
| 0 h | 5.88 ± 0.03 | 5.88 ± 0.04 | 5.88 ± 0.01 | 5.88 ± 0.02 |
| 12 h | 5.76 ± 0.01 | 5.91 ± 0.01 | 5.70 ± 0.02 | 5.66 ± 0.01 |
| 24 h | 5.79 ± 0.02 | 5.84 ± 0.01 | 5.62 ± 0.03 | 5.70 ± 0.01 |
| 48 h | 5.82 ± 0.03 | 5.59 ± 0.02 | 5.78 ± 0.04 | 5.75 ± 0.05 |
| 7 d | 5.72 ± 0.01 | 5.69 ± 0.03 | 5.40 ± 0.03 | 5.59 ± 0.03 |
| 14 d | 5.67 ± 0.01 | 5.74 ± 0.04 | 5.27 ± 0.02 | 5.60 ± 0.02 |
| 21 d | 5.88 ± 0.02 | 5.84 ± 0.01 | 5.65 ± 0.01 | 5.53 ± 0.02 |
| 28 d | 5.79 ± 0.01 | 5.70 ± 0.02 | 5.63 ± 0.01 | 5.32 ± 0.04 |
| 60 d | 5.58 ± 0.05 | 5.69 ± 0.01 | 5.70 ± 0.02 | 5.55 ± 0.03 |
| 90 d | 5.20 ± 0.02 | 5.68 ± 0.03 | 5.08 ± 0.02 | 5.24 ± 0.01 |

Table 6. pH values for formulation kept at 8 °C, 25 °C, 40 °C and 40 °C + 75% RH.

40 °C and 40 °C+ 75% RH was 5.18, 4.58, 4.29, and 4.01, respectively (Table 5).

On the other hand, pH of the samples kept at 8 °C, 25 °C, 40 °C and 40 °C + 75% RH showed a gradual decline in the pH values with a little variation with time. The pH values of samples of formulation kept at 8 °C, 25 °C, 40

°C and 40 °C + 75% RH were 5.20, 5.68, 5.08, and 5.24 at 90th day, respectively (Table 6).

The comparison of pH of base and formulation with regular time interval at different storage conditions is shown in Figs. 3A-D, respectively.

By using two-way analysis of variance (ANO-

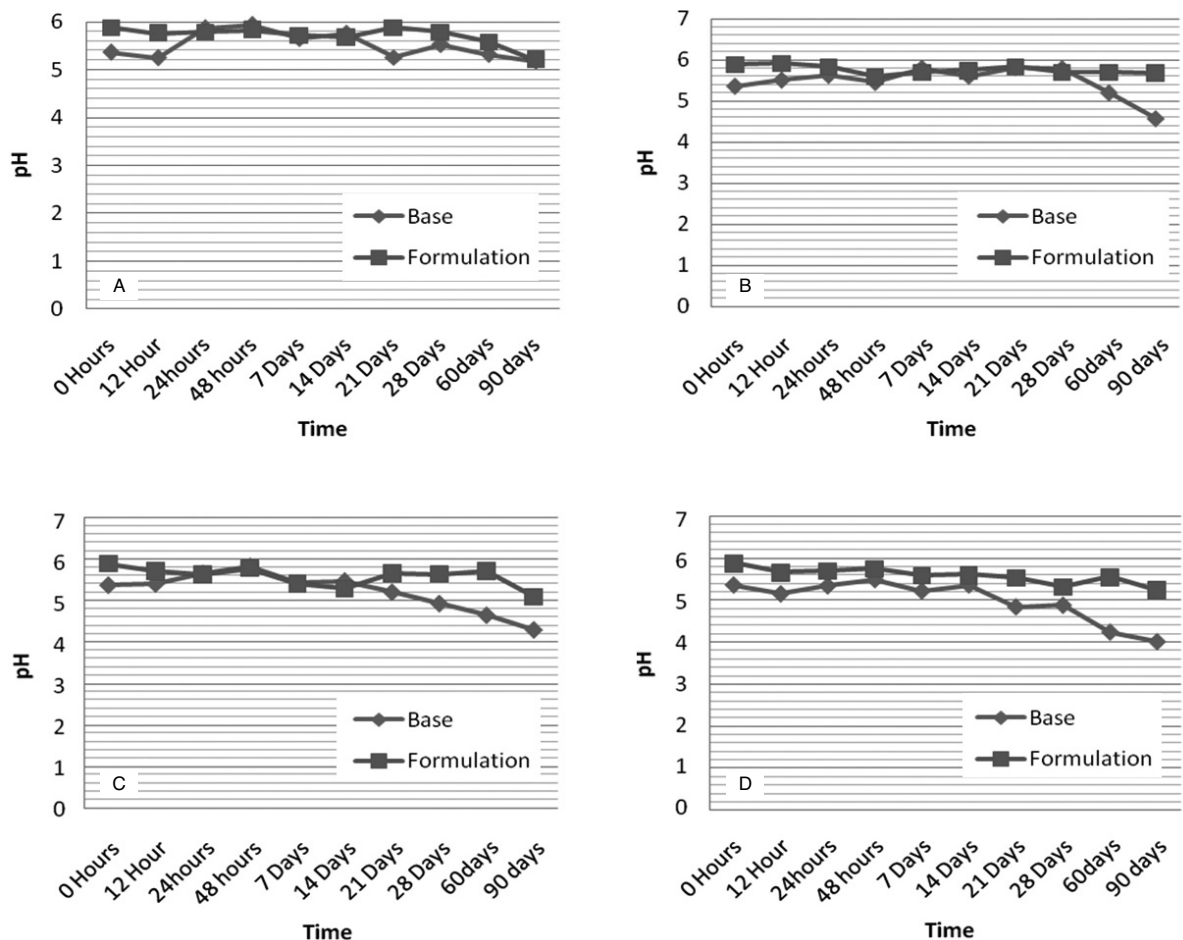


Figure 3. pH values of base and formulation kept at 8 °C (A) 25 °C (B), 40 °C (C), and 40 °C + 75% RH (D).

VA) technique at 5% level of significance, it was evident that the change in the pH of different samples of base was insignificant at different intervals of time at different temperatures but there was significant difference in the variations in the pH of different samples of formulation at different intervals of time at different temperatures. The individual average effect on pH of formulation samples with the passage of time at different temperatures was determined by applying the LSD test. It was concluded from LSD test that at different storage conditions there was insignificant change observed in pH of the base samples but in formulation samples with the passage of time, at different storage conditions significant changes were monitored in pH values. The decrease in pH is might be due to the production of highly acidic by-product from any of the acidic ingredients of the *Tamarindus indica* seeds extract such tartaric acid, acetic acid, and succinic acid ²⁵. 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 ⁶. Chemical stability performance depends upon testing at elevated temperatures and pH-profile kinetics. For the effectiveness of the cosmetic emulsions pH is a major parameter ²⁶. As the pH determined at various intervals was within the skin pH range, so the formulations can be used safely on human skin.

CONCLUSION

From the current study, we concluded that cosmetic emulsion entrapped with *Tamarindus indica* L. seeds extract in concentration of 4% showed promising stability and physicochemical characteristics in different storage conditions. The formulation provides a novel emulsion delivery system for skin rejuvenation. Further *in vivo* studies have to be done to explore this emulsion for the cosmetic market. Our research has been proved to be favorable in terms of future possible benefits of *T. indica* seeds extract in dermocosmetic products.

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