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Superlative behavior of W/O type phytocosmetic formulation's SPF (solar protection factor) in response to thixotropic and antioxidant attributes.

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Abstract

The aim of the current research work was to develop a stable herbal cosmetic formulation and to study its rheological characteristic and their impact on solar protection factor (SPF). The formulations possess good antioxidant potential and virtuous sensory attributes. An accelerated stability study was performed under variant thermal conditions for 60 days in order to evaluate any variation in droplet size and thixotropy. The droplet size of fresh formulations F1 (WSEA) and F2 (SNEA) was found to be 2.04 ± 0.577 µm and 2.31 ± 0.532 µm with thixotropic value 16964 ± 41 (D/cm^2.s) and 16471 ± 35 (D/cm^2.s) respectively. The solar protection factor at optimum thixotropic values of both formulations F1 and F2 was 3.11 ± 0.34 and 2.34 ± 0.12 respectively. At accelerated stability conditions, there was a minute reduction in the formulation consistency due to decrease in viscosity and increase in droplet size. The good SPF in these herbal cosmetic formulations was attributed to the presence of polyphenols, which protect the skin from harmful UV radiations.

Keywords: Cosmetic, W/O emulsion, Rheology, Thixotropy, SPF.

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Introduction

The acceptance and application of pharmaceutical products appreciably depend on flow properties of final formulation. Flow behavior contribute significantly from each step of pharmaceutical development process (filling, mixing, packing) to *in vivo* action [1]. The various analytical method like centrifugation and rheological studies are exercised to achieve long term physical stabilities of topical cosmetics formulation because it reduce the manufacturing time of new products. Topical delivery of drugs show skin curing action against many disorder, however the main problem with this the low penetration of active drugs at administration site [2]. Thixotropic attributes of pharmaceutical formulation influence therapeutic efficiency of drugs. In drug delivery system, modification in the rheological behavior of topical formulation result in control release of drug and enhance systemic bioavailability by augmenting retention time at site of application [3].

The term thixotropy in book of "Thixotropie" was first introduced by Freundlich. It has been noted that thixotropic material are more fluid as the applied force duration increase. The thixotropic fluid may be characterized as Newtonian or non-Newtonian. Most of the thixotropic material exhibit non-Newtonian flow like paste, ointments, cosmetics and other personal care products like lotions, creams, lipsticks, tooth paste and nail polishes [4]. It has been reported that; cosmetic product possess pseudo-plasticity or shear-rate thinning behavior, as the shear rat increase the viscosity of cream decrease. Rheological attribute are topmost cosmetic feature not only for technical but also from stunning view point. Cosmetic product performance and its sensory impute are directly relate to its rheological aspect [5].

The sunscreen formulations exhibit a pseudo-plastic behavior. They form protective layer and rapidly spread over the skin. Sunscreen's stability, film thickness, opacity and its unvaryingaction should be taken into account during formulation process. Since most of cosmetic product contain herbal extract having antioxidant power, that sure stability. The choices of sunscreen product depend on solar protection factor. In response to thixotropy, a low thixotropy result high solar protection factor [6].

Exploring the world cosmetology, the current research work was initiated, to develop stable phytoformulation and evaluate their sun protection, organoleptic and rheological behavior at different storage condition with the passage of time.

Materials and Methods

Herbal specimen collection and extraction

Berries of *W.somnifera* and *S.nigrum* were collected from vicinity of Baghdad-ul-jadeed campus, were identified by Cholistaninstitute of desert study, the Islamia University of Bahawalpur. All the samples were washed with running tape water to remove any type of impurities, fresh weighted, shaded dried for 2 weeks and weight again to determine loss on drying. Samples were grinded to powder with mechanical grinder and kept at room temperature for future analysis.

About 300 mg of each powdered sample residue macerate in 1500 ml methanol for 3 days. Filtrated with muslin cloth to remove residue and further purification was done with whatman filter paper No: 1. Filtrate were kept at room temperature till dryness and fractionated with varied non-polar and polar solvent.

Chemical reagents/instrumentation

Methanol, ethyl acetate, n-hexane was obtained from Merk (Germany). Paraffin oil, abil EM90, Homogenizer, Electrical balance (Precisa BJ-210, Switzerland), Rotary evaporator (heidolph, Co.Ltd. Japan). UV spectrophotometer (UV 4000 ORI, Germany), HPLC-DAD (Agilent Germany), Sorbex RXC8 (Agilent USA). Optical microscope (Nikon,eclipase E200), pH meter, conductivity meter, incubator, water bath, Rheological meter (Brookfield AMETEK, USA). Distilled water and all other chemicals/solvents used in research were of analytical grade and obtained from Faculty of pharmacy and alternative medicine, The Islamia University Bahawalpur, Pakistan.

Standardization of fractionated samples

Sample preparation, quantitative phytochemical screening and standardization of fractionated samples were performed [7]. Various methodologies/protocols were adopted for phyto-components quantification and their antioxidant potentials. Total flavonoids contents (TFC) from fractionated samples were quantified by adding 0.3 ml of sample in 0.15 ml of 0.5 M $NaNO_2$ followed by the addition of 0.1 ml of 0.3 M $AlCl_3.6 H_2O$, and 3.4 ml of 30% methanol. To this mixture, 1 ml of 1 M NaOH was added after 5 mint incubation at room temperature and optical density was recorded at 506 nm. Total flavonoids content expressed as µg Quercetin equivalents (QE) per mg of dry sample. For evaluation of total phenolic contents, 1 mg of each sample was mixed in 9 ml of distilled water and 1 ml of Folin-Ciocalteu reagent. The final volume of mixture was made 25 ml by adding sufficient quantity of distilled water and 10 ml of 7% Na_2CO_3. Absorbance was taken at 750 nm after 90 min incubation result stated as µg of gallic acid equivalents (GAE) per mg of dry sample.

Formulation of herbal cosmetic emulsion

After standardization and antioxidant potential evaluation of various fractions of samples; the fraction that show strong antioxidant potential and high phenolic acid contents wereselected for the development of formulation. Paraffin oil was used as oil phase and Abil EM90 (non-ionic surfactant with HLB value 5) as emulsifying agent.

The composition of aqueous phase and oil phase for emulsion was stated below (Table 1);

Briefly, accurately weighted emulsion components were heated at water-bath till temperature of both phases reached up to 75°C because temperature above 60°C reduces final droplet size of emulsion system during emulsification and also improves continuous phase uniformity. Initially the speed of homogenizer was set (2000 rpm for 15 min), then aqueous phase was carefully added into oil phase drop by drop. After 15 min the speed of homogenizer was reduced to 1500 rpm for 10 min until the emulsion consistency was achieved. The formulation was allowed to set at room temperature by further reducing the speed of homogenizer (500 rpm for 5 min).

Phase studies and sensory attribute of cosmetic formulations

Visual inspection of emulsion was done. The investigation of emulsions for any type of color change, phase separation, smell, appearance and liquefactions; was done after 0 hour and then at 8°C, 25°C, 40°C ± 75RH and 40°C in 100 ml beaker for 8 weeks.

Centrifugation test was performed according to modified method of Anchisi et al. [8], the samples were centrifuge in 40 ml centrifugal tube at 3000 rpm for 20 mint and results was reported as percent phase separation rate i.e. 100=stable, 0=instable.

According to FDA (Food and Drug Regulatory Authority) and COLIPA (The European Cosmetic Toiletry and Perfumery Association), ten (10) volunteers were used for each formulation [9]. A panel of 13 participants was included in the study for sensory evaluation of emulsion. Already coded samples were provided in randomized order to each participant for emulsion characterization and comparison. Each participant was asked to apply the formulation between

Table 1. *Represent the composition of formulation F1 and F2.*

Type	Aqueous phase Distilled water	Active	Oil phase Paraffin oil	Abil EM90
Formulation 1*	Q.s 100 %	1 mg/ml (4%)	16 %	3.5 %
Formulation 2**	Q.s 100 %	1 mg/ml (4%)	14 %	3 %

*F1=Berries of *W. somnifera* **F2=Berries of *S. nigrum* the fingers and rubbed. Following sensory parameter were examined in panel test and given 5 (high) to 1 (low).

- Visual inspection of emulsion
- Cream feeling (thick, thin, slippery, sticky)
- Skin feeling during absorption of cream (high or low force needed for cream spreadabality)
- Skin appearance after application of cream (shiny, scum).

Size Distribution of the W/O emulsion

Emulsion droplet size investigation was done using optical microscope with oil emersion lens (100X/1.25) connected to an external source. A minute quantity of formulation was diluted with continuous phase and added on glass slide with cover slip and examined under microscope. Droplets were randomly selected for size determination using Digimizerimage analyzer software.

Rheological aspect

For evaluation of physical stability of formulations therheological characterization was performed in a model DV-111 Brookfield rotational rheometer, with a cone-plate configuration and operated through Brookfield software (RHEOCALC, V2.6) [9]. Varied rheological parameter was measured at 25°C, using spindle CP41 and 0.2 g cream into assembly. The analysis was executed after 24 hours development of creams formulation and then at different storage conditions with different time interval. Ascendant and descendant rheogram curve were obtained with progressively increasing (10-55 rpm) and decreasing (55-10 rpm) spindle speed. The area under the segmented curve was calculated by Graph Pad Prism software version 5.01. The consistency index, flow index and yield stress were mathematically calculated by power law rheological model of Ostwald-de waele.

$$\tau = K\gamma^n$$

Where, τ =Shear Rate, K=Consistency Factor, γ =Shear Rate and n=Flow Behavior Index.

SPF measurement

Solar protection of formulations was investigated by modified methodology of Dutra *et al*., [10]. Briefly, 1.0 g of sample of each formulation was diluted with dimethyl sulfoxide (DMSO) in 250 ml volumetric flask. Filtered it, through filter paper, discard 1st 10 ml. 5 ml of aliquot was taken and further dilutions was made with DMSO in 50 ml volumetric flask. Diluted, 5.0 ml was transferred to 25 ml volumetric flask and diluted with sufficient quantity of DMSO. The absorbance of sample was taken at 295 nm-320 nm. And SPF was determined by Mansur equation.

$$SPF = CF \times \sum_{290\text{-}320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Where, CF=Correction Factor, EE=Erythmogenic Effect, the value of EE (λ) × I.

Results and Discussion

Texture and cosmetic properties of the creams

Panel tests of thirteen participants were included in sensory evaluation of fresh creams. In sensory evaluation of fresh formulations, the four basic characteristic of cosmetic cream was observed by panelist i.e., cream appearance (thick or thin), feeling (sticky or slippery), skin feeling during (force needed) and after application (shiny or sum). From the results of 13 participants this will be observe that formulation F1 is thick, slippery, non-sticky, no force is required during rubbing and did not give shiny look on skin after application. Similarly for F2 results indicate that the formulation is thin, slippery, non-sticky, no force is required during rubbing and did not give shiny look on skin after application.

Accelerated stability studies

Organoleptic attribute of formulation F1 (WSEA fraction) and F2 (SNEA fraction) at different thermal conditions with altered time interval. Variations in color, appearance, smell and spreadabality were analyzed during study period of two month. The formulations remain stable throughout the study with respect to no change formation in color and smell. However, at high storage temperature i.e. at 40 and 40+75 RH, after 60 days minute deviations were observed in appearance and spreadabality of both formulations.

Droplet size distribution

The droplet size of cream formulation is not only the stability determining factor but also explain its rheological aspects [11]. The droplet size of emulsion systems was determined after 0 hours of development and after 60 days storage at various thermal conditions. Droplets size, of fresh formulations of berries of *W.somnifera* ethyl acetate fraction (F1) and *S. nigrum* berries ethyl acetate fraction (F2) was found to be 2.04 ± 0.577 µm and 2.31 ± 0.532 µm respectively (Figures 1 and 2). Emulsion droplets size analysis after 60 days, at high storage temperature was confirmed that mean droplet size of both formulation F1 and F2 was increased,i.e.7.21 ± 3.532 µm and 6.40 ± 2.927 µm respectively (Figure 2). The study show that the droplet size of both formulation increases as compared to fresh formulation, the increase in the droplet size of emulsion system will result in decrease the consistency index and viscosity on storage [12].

Rheological attributes and solar protection

Rheological behavior of emulsion continuous phase and droplet size distribution, particle-particle interaction, internal viscosity and droplet concentration/size are the factors that determine the basic rheological characteristic of emulsion system [13]. Rheological behaviors of cosmetic formulations were examined 0 hour after preparations and then after 7, 15, 30 and 60 days at various thermal conditions. With Power law Model, Casson Model and IPC past Model; the value of apparent viscosities and consistency index was calculated (Figure 3). The area under the ascendant and descendent rheogram curves (thixotropy) and SPF (solar protection

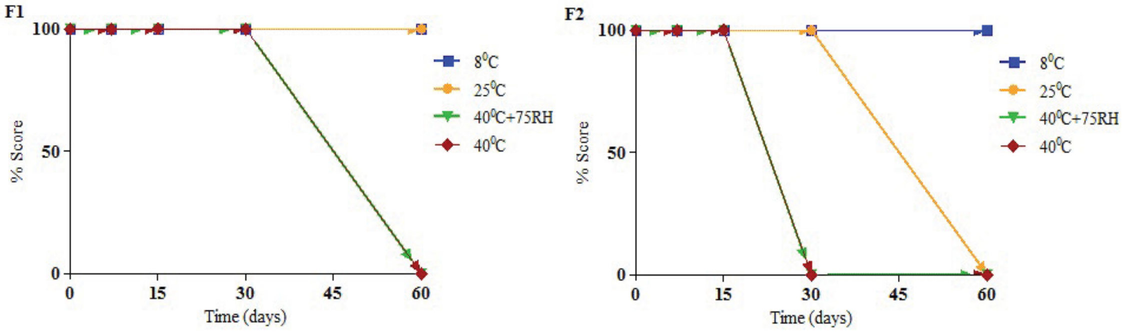

Figure 1. *Phase separation study of formulations at accelerated conditions. F1 formulation contained active Withania somnifera ethyl acetate fraction, F2 formulation contained active Solanum nigrum ethyl acetate fraction, RH, relative humidity. The data was presented as percent, 100=stable and 0=instable, on the scale of 0-100 and gradually phase separation occur from 100-0 at different temperatures.*

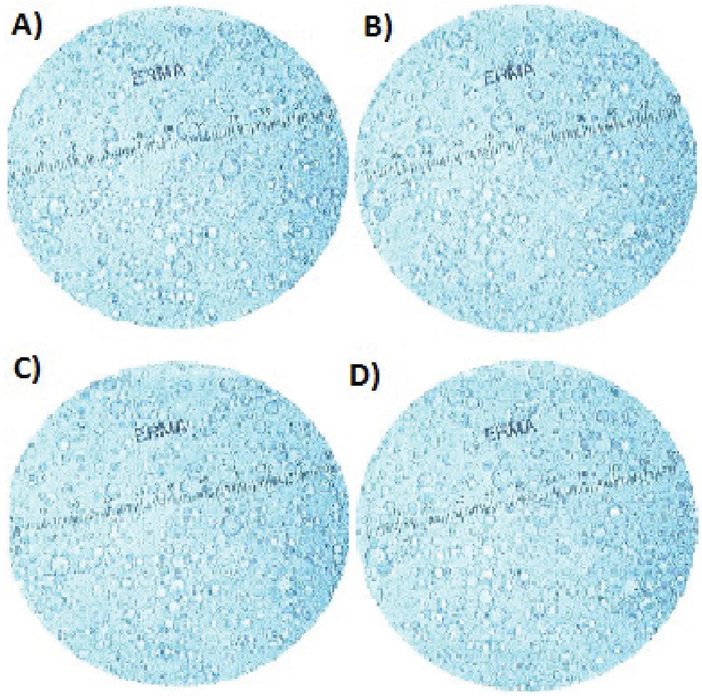

Figure 2. *Droplet size distribution of fresh formulation, A) Formulation F1 (WSEA), mean droplet size is 2.04 ± 0.577 µm (n=80), B) Formulation F2 (SNEA), mean droplet size is 2.31 ± 0.532 µm (n=80). Scale=100 µm, C) Formulation F1 (WSEA), mean droplet size is 7.21 ± 3.532 µm (n=20), Min=1.82 µm and Max=12.98 µm after 60 days at 40ºC, D) Formulation F2 (SNEA), mean droplet size is 6.40 ± 2.927 µm (n=20), Min=1.55 µm and Max=12.60 µm, Scale=100 µm after 60 days at 40ºC.*

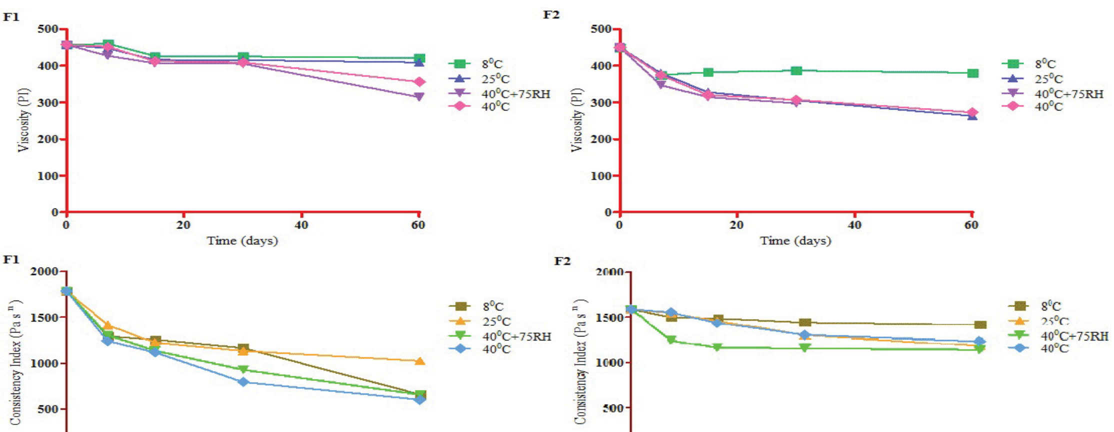

Figure 3. *Graphs represent the reduction in the Viscosity and Consistency Index of cosmetic formulation at various thermal conditions, A) F1 formulation contained active Withania somnifera ethyl acetate fraction at different storage conditions for two month, B) F2 formulation contained active Solanum nigrum ethyl acetate fraction at different storage conditions for two month.*

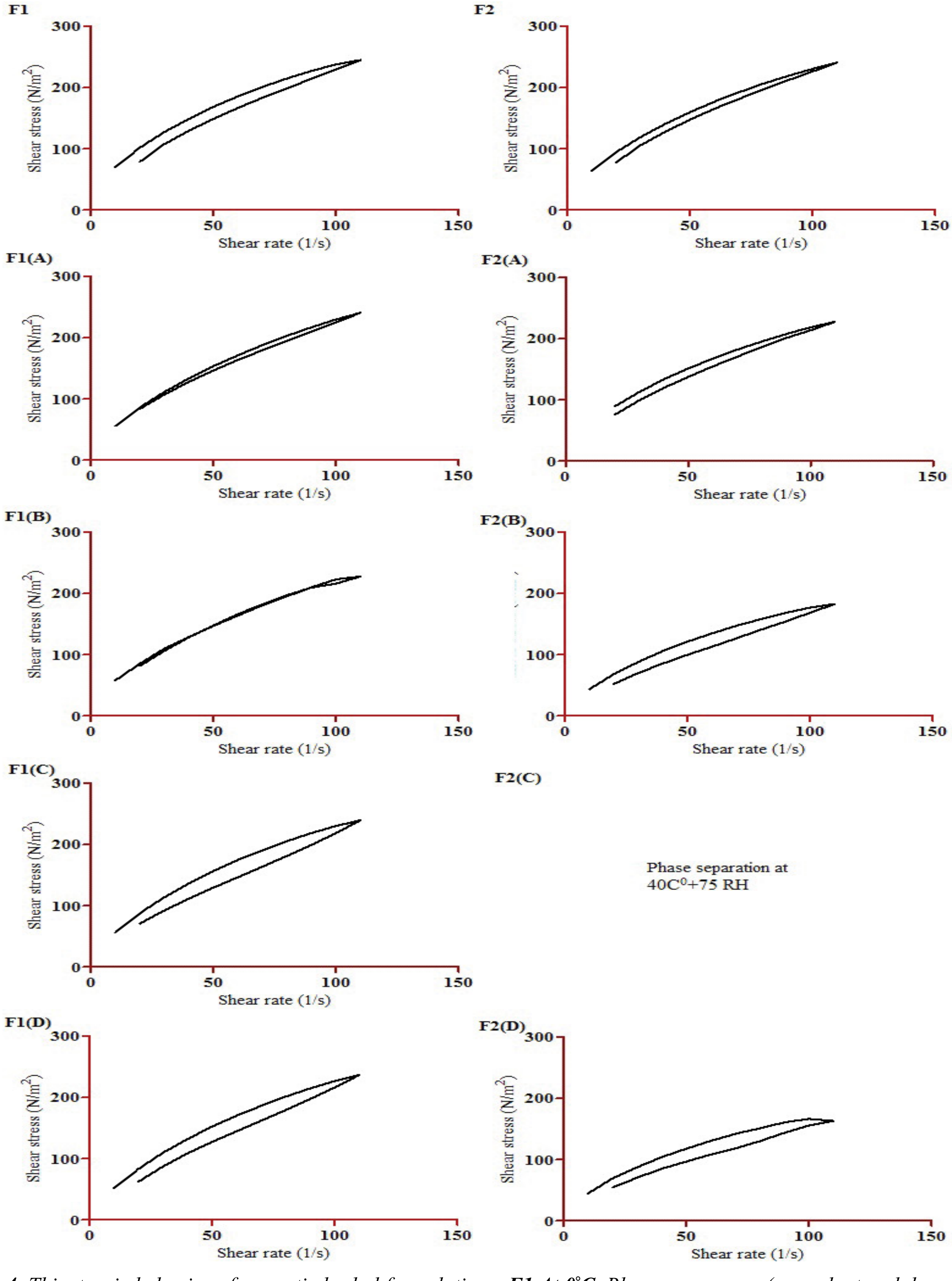

***Figure 4**. Thixotropic behavior of cosmetic herbal formulations, **F1 At 0°C**, Rheogram curves (ascendant and descendent) of formulation contained ethyl acetate fraction of Withaniasomnifera berries, First X=10.00, First Y=110.0, Peak X=110.0 and Peak Y=245.4, Area under the segmented curves 16964 ± 41.3. **F2 At 0°C**, Thixotropic behavior of formulation contained ethyl acetate fraction of Solanumnigrum berries, First X=10.00, First Y=110.0, Peak X=110.0 and Peak Y=241.42, Area under the segmented curves 16471 ± 39.1 (D/cm^2.s). **F1(A) At 8°C**, First X=10.00, First Y=110.0, Peak X=110.0 and Peak Y=241.2, Area under the segmented curves 16175 ± 35.2 (D/cm^2.s). **F2(A) At 8°C**, First X=20.00, First Y=110.0, Peak X=110.0 and Peak Y=227.9, Area under the segmented curves 14836 ± 34.7. **F1(B) At 25°C**, First X=10.00, First Y=110.0, Peak X=110.0 and Peak Y=228.2, Area under the segmented curves 15853 ± 34.98. **F2(B) At 25°C**, First X=10.00, First Y=110.0, Peak X=110.0 and Peak Y=183.22, Area under the segmented curves 12070 ± 32.98 (D/cm^2.s). **F1(C) At 40°C +75RH**, First X=10.00, First Y=110.0, Peak X=110.0 and Peak Y=240.00, Area under the segmented curves 15652 ± 29.81. **F1(D) At 40°C**, First X=10.00, First Y=110.0, Peak X=110.0 and Peak Y=237.00, Area under the segmented curves 15290 ± 36.91 (D/cm^2.s). **F2(D) At 40°C**, First X=10.00, First Y=110.0, Peak X=110.0 and Peak Y=163.00, Area under the segmented curves 11536 ± 26.89.*

Table 2. *Thixotropic and SPF behavior of herbal cosmetic formulation at different storage conditions.*

	Thixotropy							
	F1				**F2**			
	8°C	25°C	40°C+75RH	40°C	8°C	25⁰C	40°C+75RH	40°C
Fresh	16964 ± 41	16964 ± 41	16964 ± 41	16964 ± 41	16471 ± 35	16471 ± 35	16471 ± 35	16471 ± 35
60(days)	16175 ± 39	15853 ± 34	15290 ± 37	15652 ± 29	14836 ± 34	12070 ± 36	-	11536 ± 26
	SPF							
	F1				**F2**			
	8°C	25°C	40°C+75RH	40°C	8°C	25°C	40°C+75RH	40°C
Fresh	3.11 ± 0.34	3.11 ± 0.34	3.1 ± 0.34	3.1 ± 0.34	2.3 ± 0.12	2.3 ± 0.12	2.3 ± 0.12	2.3 ± 0.12
60(days)	1.23 ± 0.10	1.34 ± 0.21	1.1 ± 0.12	1.1 ± 0.11	1.1 ± 0.01	0.9 ± 0.11		1.23

Each value is presented as mean ± SD (*n*=3).SPF: Solar Protection Factor, RH: Relative Humidity; F1:Formulation with WSEA as Active; F2: Formulation with SNEA as Active

Table 3. *Correlation between SPF of cosmetic formulations with Thixotropy and viscosity.*

SPF	R^2							
	F1				**F2**			
	8°C	25°C	40°C +75RH	40°C	8°C	25°C	40°C +75RH	40°C
Thixotropy	0.9989[a]	0.9977[b]	0.9946[b]	0.9968[b]	0.9946[b]	0.9546[c]	-	0.9415[c]
Viscosity	0.9966[b]	0.9938[b]	0.9353[c]	0.9698[c]	0.9862[b]	0.8778[ns]	-	0.8915[ns]

SPF: Solar Protection Factor, RH: Relative Humidity, F1: Formulation with WSEA as Active, F2: Formulation with SNEA as active, Significance of linear correlation: [a]$p<0.05$), [b]$p<0.01$, [c]$p<0.001$. ns: Not Significant

factor) was determined 0 hours formulations development and after 60 days at different storage conditions. The solar protection factor values for fresh F1 and F2 formulations was proven to 3.11 ± 0.34 and 2.34 ± 0.12 respectively at optimum thixotropic value of 16964 ± 41 (D/cm^2.s) and 16471 ± 35(D/cm^2.s) (Table 2). The formulations with optimum thixotropy values have high solar protection, if the thixotropic value lies below the optimum value than there is poor spreadabality of cream and if this value lies above the optimum value then there is structural breakdown occur in the emulsion system due unevenly distributed film (Figure 4) [14]. Antioxidant compounds such as phenols and flavonoids are added in commercially available sunscreen, in order to protect from photo damage by harmful UV radiations and to augment solar protection effect of these sunscreens [15].

The viscosity of fresh formulation F1 and F2 was calculated as 457 (P) and 450 (P) respectively. The viscosity of fresh formulations was gradually reduced with increasing shear stress and regular increasing shear rate. At the lapse of 60 days at various storage conditions (Table 3); the viscosity, flow index and consistency index of formulations decrease which show the sign of instability at accelerated storage conditions.

Conclusion

In the current work, we observed that herbal cosmetic formulation exhibit strong antioxidant activity with good solar protection values. We observed that only at accelerated thermal conditions there will be miner change in consistency of formulation was detected, due increase in droplet size. However the thixotropic values of formulation not much effected at storage conditions and this will influence therapeutic efficiency of drugs, provide control release of herbal antioxidant ingredient form emulsion system and boost systemic bioavailability by amplifying retention time at site of application.

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Conflict of Interest

Author declared no conflict of interest.

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