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Vicissitudes in polyphenolic extract-based high internal phase creams (HIPCs)- effect of storage temperature dependent characteristics

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Abstract: The purpose of this investigation was to evaluate the vicissitudes in polyphenolic extract- based high internal phase creams (HIPCs) and effect of storage temperature dependent characteristics. Rheological parameters, that is, power law and IPC analysis with its physical characteristics were exploredat different storage temperatures (8°, 25°, 40° and 40° with 75% relative humidity- RH) with different time intervals up to 2 months of newly formulated poly-phenolic extract- based high internal phase cream and its comparison with base. Polyphenolic- based HIPCs showed non-Newtonian- pseudo plastic tendencies in vicissitudes with time and storage temperatures. Data analysis with Power Law and IPC paste was found to fit to all the rheograms. Flow index, shear sensitivity factor, consistency Index and 10 RPM of freshly prepared HIPCs with and without encapsulated polyphenolic extract were found to be 0.5,0.53, 386.4 cP, and 432.9 cP, respectively.The viscosities were fallen with rise in shear stress.There was no change in color, electrical conductivity, liquefaction and phase separation after centrifugation in any sample of polyphenolic extract– based HIPCs and its base. Polyphenolic- based extract HIPCs behaved non-Newtonian- pseudo plastic tendencies and showed stability up to 2 months and can be directed absolutely to shield skin against ultraviolet radiation (UV) intervened oxidative mutilation.

Keywords: Formulation, rheology, storage temperature, high internal phase creams.

INTRODUCTION

High internal phase creams (HIPCs) are disperse systems holding a high volume of disperse phase. Consideration on the reasons that control the development and stability of HIPCs is much immature with existing knowledge (Kizling *et al.,* 2006; Gallegos and Franco, 1999).

The characterizations of rheology are vital to control flow and deform properties in various areas, that is, industrial chemistry, cosmetic chemistry, pharmaceuticals and polymer chemistry, etc. (Baby *et al.,* 2008; Goodwin *et al.,* 2008). The developments of changes in the rheological properties of creams embody significant forewarnings failure of the product (Clement *et al.,* 2000; Korhonen *et al.,* 2001).

The controlling characteristics and the principles for rheological parameters vary on the stress and the extent of stress applied (Korhonen *et al.,* 2000).

Amalgamation of antioxidants and phenolic compounds via topical applications to protect skin against oxidative damage has reported a flourishing strategy (Ali and Akhtar, 2015; Alena and Jitka, 2003).

Antioxidants are of immeasurable significance due to their influence in principal bio-industrial processes. Overall, antioxidant compounds possess anti-inflammation, anticancer, anti-cardiovascular, anti-proliferative, anti-oxidative and many other activities (Reis *et al.,* 2007).

Cannabis sativa (Cannabaceae) is inborn to Central Asia like India, Pakistan, Sri Lanka and China with long cultivation in Asia, China, and Europe (Zuardi *et al*., 2010).

Phyto-chemistry of *Cannabis* characterize the chemical classes like phenolic compounds, phyto-cannabinoids, mono- and sesquiterpenes, hydrocarbons, steroids, flavonoids, nitrogenous compounds, sugars and amino acids, among others (Radwan, 2008; Elsohly and Slade, 2005).

Recent developments on the medical remedies of Cannabis sativa (Cannabaceae) has been commenced, mostly Asian and European countries move towards a more non-interventionist on the exploitation of Cannabis as a treatment (Hazekamp *et al.,* 2004; Oomah *et al.,* 2002; Baker *et al.,* 2003; Yotoriyama *et al.,* 2005).

Our investigation was to evaluate the vicissitudes in polyphenolic extract- based high internal phase creams (HIPCs) and effect of storage temperature dependent characteristics.

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MATERIALS AND METHODS

Seeds of *Cannabis sativa* were purchased from local market of Islamabad, Pakistan on August, 2011. *Cannabis* seeds were identified by Cholistan Institute of Desert Studies and specimen (Voucher No. CS-SD-01-11-32) was deposited by Herbarium, the Islamia University of Bahawalpur, Bahawalpur- Pakistan. ABIL EM 90 was obtained from Franken Chemicals Germany, Paraffin oil was procured from Merck, Germany and Methanol was procured from BDH, England.

Preparation of the polyphenolic extract

The crushed plant material (40 g) was extracted with solvent-aqueous methanol (methanol: water, 80:20 v/v) (1L) – in mechanical mixer for 6 h at room temperature (Euro-Star, IKA D 230, Germany). The extract through what man No. 1 filter paper by filtering was removed from the residues. The residues with the same fresh solvent were extracted twice and extracts combined. The mixed extracts were condensed under reduced pressure at 45°, using a rotary evaporator (Eyela, Co. Ltd. Japan) and freed of solvent up to one tenth. The concentrated polyphenolic extract was stored for further experiment at -4° (Sultana *et al*., 2009).

Preparation of the polyphenolic extract- based high internal phase creams

2% ABIL EM 90, 14% Paraffin oil, 3% polyphenolic extract, 1% fragrance and deionized water were consumed for formulation. Both oily phase and aqueous phase were heated up to $75^{\circ}\pm1^{\circ}$ and then combined by adding polyphenolic extract and fragrance using homogenizer (Euro-Star, IKAD 230, Germany). The same method was adopted to prepare the base.

Determination of anti-oxidant activities

Anti-oxidant activities of the polyphenolic extract alone and after addition in the extract cream were determined. The antioxidant capability was evaluated by consuming a methanol solution of DPPH. The DPPH discloses absorbency at 517 nm at maximum. The DPPH stable free radical was used to determine free radical scavenging of extract. In 5 micro liters of aqueous methanolic polyphenolic extract, added DPPH to fabricate the volume up to 100 µl in 96 well plates. Admixed the contents and incubated for 30 minutes at 37° and determined the optical density at 517 nm. Ascorbic acid, as standard, had a strong antioxidant property that's why it was used as standard to evaluate the antioxidant activity of substances (Sultana *et al*., 2009). Experiments were done in triplicates. Results were taken as mean and standard error of mean of three independent experiments.

% DPPH scavenging activity = (100 - OD of test sample/ OD of controlled x100)

Assessment of rheological characteristics

Up to two months, HIPCs (polyphenolic extract- based HIPCs and its base) were submitted to storage temperatures at 8°, 25°,40° and 40° with 75% RH. Resulting samples were used for the evaluation of viscosity and rheological readings at the initial time, then after one and two months. The viscosities and rheological behavior of HIPCs were established using a rotational rheometer with a cone-plate configuration (Brookfield DV-III Ultra) with a CP41 spindle. All the rheological tests were done at 25° with additional a Brookfield software program, Rheocalc V2.6. Nearly0.2 g samples and a constant temperature of 25° were exercised for the tests. Tests were repeated three times, each containing 10 values of shear rate. Increasing shear stresses were operated to the samples. Ensuing the purpose of the flow type, flow curves were fitted to the mathematical models. Increased shear stresses were operated on the samples and the shear rates and changes in viscosities were noted.

Data obtained were investigated by using Brookfield Software Rheocalc version (2.6). IPC Paste and Power Law (PL) math models numerically and graphically analyze the behavior of data sets.

Power Law

The Power Law equation is $\tau = kD^n$ *where*:
τ= Shear Stress
D = Yield Stress (stress at zero shear rate)
k= Plastic Viscosity
n = Shear Rate
The calculated parameters for this model are:
Flow Index (no units)
Consistency Index (cP)
Confidence of Fit (%)

IPC Paste Analysis

This method is intended to calculate the Shear Sensitivity Factor and the 10 RPM Viscosity value of creams. The Paste equation is: $\eta = kR^n$ where:
η = Viscosity (cP)
k= Consistency Multiplier
R = Rotational Speed (RPM)
n = Shear Sensitivity Factor
The calculated parameters for this model are:
Shear Sensitivity Factor (no units)
10 RPM Viscosity (cP)
Confidence of Fit (%)

Physical assessment

Color, conductivity, liquefaction, centrifugation and pH were recurred for creams after 24 h, 7, 14, 21, 28, 42 and 54 d of preparation. Electrical conductivity using conductivity meter (WTW COND-197i, Germany) and centrifugation using Centrifuge Machine (Hettich EBA 20, Germany) were accomplished. The pH of creams was calculated using pH-Meter (WTW pH-197i, Germany). The centrifugal tests were implemented at 5000 rpm for 10 minutes by retaining few grams of sample in centrifugal tubes which were disposable and stoppered.

STATISTICAL ANALYSIS

SPSS 17.0 was used for data analysis on the computer by using the ANOVA and Microsoft Excel version 2016 was used for calculating means.

RESULTS

The antioxidant capability of polyphenolic extract and after addition of polyphenolic extract of the cream was found to be 87% and 79%, respectively. Rheological characteristics of both polyphenolic extract- based HIPCs and its base at different storage temperatures up to two months have been given in Table 1, 2 and 3. Rheograms of the HIPCs have been shown in Figure 1 to 5. Rheograms of shear stress versus shear rate were obtained. Power Law was achieved to fit to all the rheograms and the confidences of fit were found to be in the limit of 93.9-99.9 %. IPC paste provided the data of confidence of fit 93.9-99.9 %. Viscosities of both polyphenolic extract- based HIPCs and its base are presented in Table 4 and 5. The colors of freshly prepared polyphenolic extract- based HIPCs and its base were white. There was no change in color of any samples at different storage temperatures during the study period. In this study, the pH of polyphenolic extract- based HIPCs and its base were 4.92 and 4.61, respectively. All tested formulations showed almost no significant effect on the physiological pH-value after applied ANOVA between the skin areas with formulations and the blank area. There was no phase separation after centrifugation, no conductivity and no liquefaction found in any of the samples of base and HIPCs kept at 8°, 25° and 40° + 75% RH.

DISCUSSION

The DPPH assay is extensively used for the extent of free radical scavenging capability. The extract being antioxidant is correlated to flavanoids, proteins, tannins and reducing sugars. The phenolic compounds are reported as scavengers of free radicals (Aneta and Jan, 2007). The stalks, leaves, flowers, and seeds of the plant contain cannabinoids. The THC content varies immensely between different sources of cannabis (Ashton, 2012). Then the biochemical ingredients mostly accountable for antiradical activity of hempseed extract are secluded and recognized as N- trans-caffeoyltyramine and cannabisin B reported by T Chen *et al* (Chen *et al*., 2012). The polyphenolic extract- based HIPCs illustrated lower H-donor potential. The formulation components present in the reaction mixture followed lower H-donor potential in polyphenolic extract- based HIPCs. However, the DPPH scavenging was assessed by spectroscopy, the HIPCs ingredients may intervene with the antioxidant capacity (Huang *et al*., 1994).

The rheograms of all HIPCs showed non-Newtonian behavior, with flow index less than 1 which is an attractive rheological property in these preparations redirecting their pseudo plastic tendency. However, our findings indicated that HIPCs had better consistency and rheological results as compared to base. HIPCs with a pseudo plastic flow produce a coherent film protecting the skin surface. This feature is advantageous and necessary for an improved phenolic antioxidant fortification of the skin surface. The aim of pseudo plastic flow may be due to the developing crumbling of the internal structure of the creams, under increasing shear, and its later renovation by means of Brownian movement (Gaspar and Maia Campos, 2003). However, the silicone emulsifier (silicone polymer) ropes a product the more stable and dependable. This generates a system with durable interfacial film which may be due to steric crowding (Goodwin *et al.,* 2008).

Although flow indexes were changed by stress and consistency indexes of polyphenolic extract- based HIPCs were also increased (Table 1, 2 and 3). It is likely that this was due to the interaction of polyphenolic compounds having antioxidant activity and the vehicle polymer. It is established by an artifact when the decomposition

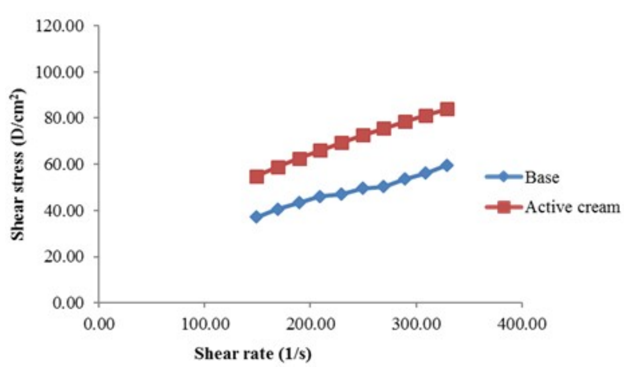

Fig. 1: Rheogram of freshly prepared base and polyphenolic extract- based HIPCs. Analyses were performed at 25°.

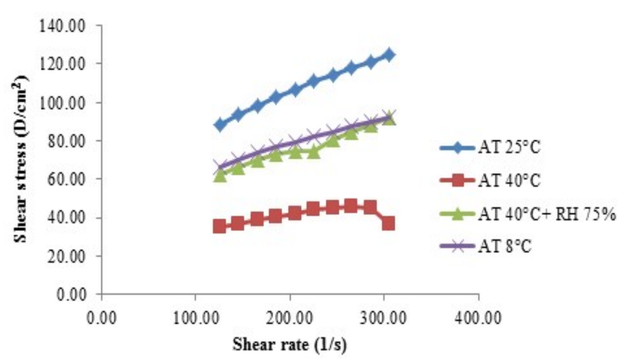

Fig. 2: Rheogram of base at fourth week kept at 8°, 25°, 40° and 40° + RH 75%

products were studied by mass spectrometry (Guaratini and Gianeti, 2006). Various factors may be subjective on the stability of system and it is testified that electrolytes in the internal/aqueous phase of concentrated W/O cream affectedly improved cream stability. The electrolytes seem to progress the stability of these water-in-oil creams by increasing the resistance of the water droplets to coalescence (Goodwin *et al.,* 2008).

Viscosities were found to decrease in parallel to the increase in shear stress. It was found that viscosities of the freshly prepared base decreased with increase in the shear stress from 3302.4 cP to 2460.3 cP and also the samples of base at 25°, 40°, 40° + RH 75% and 8° after one month and two months was found to be the same behavior. Viscosity of freshly prepared polyphenolic extract- based HIPCs was found to be 3256.6 cP which was decreased to 2548.1 cP by increasing shear stress and also the sample of polyphenolic extract- based HIPCs at 25°, 40°, 40° + RH 75% and 8° after one month and two months to be the same behavior. It was also concluded that viscosities of polyphenolic extract- based HIPCs was in acceptable range.

No change in color may be accredited to different factors that associate to stability comprising the components of oil phase, paraffin oil and ABIL EM 90 which was colorless, transparent and non-toxic liquids. Silicone surfactants exhibited characteristic properties which fashioned their use very appealing. It has been reported that non-ionic silicone surfactants get better the visual characteristics by removing high melting point waxes (Avendano-Gomez *et al.,* 2005).

The pH of formulation is within the range of skin pH and suitable for skin protection from toxic substances (Matousek *et al.,* 2003). Accordingly, the determination of the pH symbolizes an imperative factor of safety for the application of cosmetic formulations. Therefore, cosmetic formulations should not modify the physiological pH (Swatschek *et al.,* 2002).

Stability of creams is dependent on centrifugation which is one of the most physicochemical factor (Goodwin *et al.,* 2008). There was no phase separation after centrifugation in any of the samples of base and HIPCs kept at 8°, 25° and 40° + 75% RH. However, slight phase separation was examined for the samples of both polyphenolic extract- based HIPCs and its base kept at 40° after 45 days of the study period. The insistence of the surfactant is completely controlled by its surface activity to avoid coalescence of the dispersed water phase. Surfactant molecules accumulate and adsorb on the surface of the internal phase and decreases interfacial tension between oil and aqueous phases. Decisively, they offer cream stability against flocculation and coalescence of the internal phase (Choi *et al*., 2009; Muguet, 2001).

Conductivity is assessment of degree of free water and free ions in cream formulation. High or low conductivity measurements show that there is less or more lamellar water and more free water in the creams, which can be realized as a decrease or increase in the consistency of creams, respectively (Korhonen *et al.,* 2000; Kizling *et al.,* 2006). There was no electrical conductivity noted in any sample of polyphenolic extract- based HIPCs and its base till the end of study period. This is due to the cream was of W/O type and oil being the external phase provides no route of current.

There was no liquefaction in any of the samples kept at 8° and 25°. The samples were stable at 8°, 25°, but slight phase separation in the sample of base occurred at 40° and 40°+75% RH on 55th day of observation whereas the polyphenolic extract- based HIPCs was stable. This may be due to the antimicrobial properties of polyphenolics which protect the cream from microbial contamination and degradation.

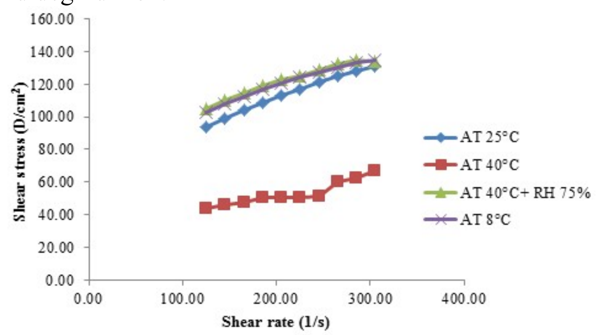

Fig. 3: Rheogram of base at eighth week kept at 8°, 25°, 40° and 40°+ RH 75%

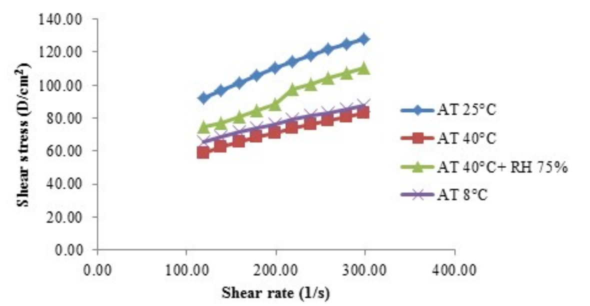

Fig. 4: Rheogram of polyphenolic extract- based HIPCs at fourth week kept at 8°, 25°, 40° and 40° + RH 75%

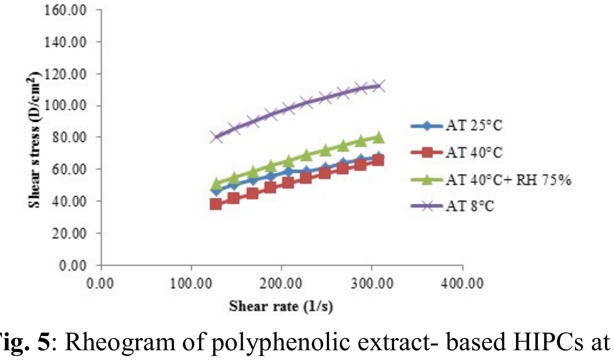

Fig. 5: Rheogram of polyphenolic extract- based HIPCs at eighth week kept at 8°, 25°, 40° and 40° + RH 75%

Table 1: Rheological parameters at freshly prepared base and polyphenolic extract- based HIPCs.

Model	Rheological parameter	Base	polyphenolic extract- based HIPCs
Power Law	Consistency Index (cP)	386.4	432.9
	Flow Index	0.50	0.53
	Confidence of fit (%)	99	99.8
IPC Paste	10 RPM Viscosity (cP)	86	105.9
	Shear Sensitivity	0.50	0.47
	Confidence of fit (%)	99	99.8

CONCLUSION

Polyphenolic- based HIPCs showed stability with respect to time and storage conditions following characteristics of creams. The pseudo plastic structure is supposed to be able to sustain structural stability and resistance to external forces for specific period. In addition, can be directed absolutely to shield skin against ultraviolet radiation (UV) intervened oxidative mutilation.

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Table 2: Rheological parameters at fourth weeks of base and polyphenolic extract- based HIPCs kept at 8°, 25°, 40° and 40°+ RH 75%.

Model	Rheological parameter	At 8°		At 25°		At 40°		At 40° + RH 75%	
		Base	polyphenolic extract- based HIPCs	Base	polyphenolic extract- based HIPCs	Base	polyphenolic extract- based HIPCs	Base	polyphenolic extract- based HIPCs
Power Law	Consistency Index (cP)	425	714.1	486.2	656.1	709.6	394.6	397.4	250.2
	Flow Index	0.51	0.43	0.54	0.50	0.30	0.52	0.51	0.64
	Confidence of fit (%)	99.7	99.9	99.6	99.8	93.9	99.7	94.1	98.5
IPC Paste	10 RPM Viscosity (cP)	99.1	128.	124.1	148.4	86.9	93.2	92.2	85.6
	Shear Sensitivity	0.49	0.57	0.46	0.50	0.70	0.48	0.49	0.36
	Confidence of fit (%)	99.7	99.9	99.6	99.8	93.9	99.7	94.1	98.5

Table 3: Rheological parameters at eighth weeks of base and polyphenolic extract- based HIPCs kept at 8°, 25°, 40° and 40°+ RH 75%

Model	Rheological parameter	At 8°		At 25°		At 40°		At 40° + RH 75%	
		Base	polyphenolic extract- based HIPCs	Base	polyphenolic extract- based HIPCs	Base	polyphenolic extract- based HIPCs	Base	polyphenolic extract- based HIPCs
Power Law	Consistency Index (cP)	476	1011	547.3	818.6	148.5	263.4	125	431.5
	Flow Index	0.46	0.44	0.53	0.40	0.63	0.59	0.40	0.53
	Confidence of fit (%)	99.6	99.5	99.7	99.3	96.1	99.9	99.3	99.8
IPC Paste	10 RPM Viscosity (cP)	95	187	135.6	136	48.5	76.5	208.5	106.2
	Shear Sensitivity	0.54	0.56	0.47	0.60	0.37	0.41	0.60	0.47
	Confidence of fit (%)	99.6	99.5	99.7	99.3	96.1	99.9	99.3	99.8

Table 4: Viscosities (cP) of base at different storage temperatures and time intervals

Sr. No.	Fresh	At 8°		At 25 °		At 40°		At 40 ° + 75%RH	
		4 weeks	8 weeks	4 weeks	8 weeks	4 weeks	8 weeks	4 weeks	8 weeks
1	3302.4	3950.9	3362.8	5277.9	5670.0	2593.7	2005.6	6363.6	3694.5
2	3194.1	3783.6	3221.6	5113.4	5442.4	2495.0	1932.9	6073.0	3564.3
3	3066.2	3656.8	3078.8	4926.0	5240.2	2349.9	1859.8	5805.7	3455.7
4	2957.9	3514.7	2934.7	4755.9	5045.9	2308.3	1786.3	5544.7	3352.3
5	2789.7	3382.1	2811.3	4588.5	4890.1	2165.0	1755.7	5310.2	3188.3
6	2704.3	3267.2	2724.4	4443.5	4735.0	2010.6	1709.0	5066.8	2965.7
7	2563.5	3166.7	2629.5	4307.1	4589.9	1922.6	1649.3	4872.6	2971.6
8	2536.9	3069.1	2545.8	4186.8	4452.9	2120.0	1578.9	4727.9	2971.6
9	2479.7	2982.4	2479.7	4063.1	4322.8	2086.0	1449.3	4549.0	2948.9
10	2460.3	2896.9	2373.0	3960.4	4198.5	2127.0	1103.2	4293.7	2896.9

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