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Physico-Chemical Stability Studies of Neem (*Azadirachta indica*) Seed Oil Cream (Pp.1-11)

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Abstract

Neem oil cream is a semi solid formulation that is being developed for its therapeutic activity dermatologically. The need for stability of the new formulation over the period of its shelf life remains a sacred factor. In the present study, neem oil had been extracted from the ripe seed of Azadirachta indica (A. juss) plant. The oil was formulated using vanishing cream base because of its cosmetic advantage at concentrations (0% $^{w}/_{w}$ *– 10.0%* $^{w}/_{w}$*) with 0%* $^{w}/_{w}$ *serving as control formulation. The formulations at storage temperature* $25^{0}C$ *–* $30^{0}C$ *were observed for physical changes using basic parameters over a period of 12 months representing long term stability studies approach. Chemically, the formulations were investigated for changes in pH, viscosity and specific gravity during the period of study. The result shows that neem oil cream formulations maintain its physical and chemical integrity throughout the entire period of study concern. Hence, stability is not a problem for neem oil in a vanishing cream base formulation.*

Keywords: Neem oil, Stability, Shelf life, Formulations.

Introduction

Azadirachta indica A. Juss, synonymous with *Melia azadirachta* and *Melia indica* (A. Juss) belongs to the family meliaceae. This plant is indigenous to the Indo-Pakistan subcontinent (Vander *et. al.* 1987), although it is now widely distributed in many countries of the world, it is believed that Indians migrating to African countries introduced it into that continent. Recently, Aremu *et. al.* 2008 in their review article reported on earlier indications of neem seed oil medicinally proven severally in the literature, repellant action against *Aedes aegypti* mosquitoes (Hati *et. al,* 1995), anti candidal activity (Charmaine *et. al,* 2005), antipyretic effect (Okpanyi and Ezeukwu, 1981) etc. Aremu *et. al.* 2008 also revealed in their article the need for a delivery design of neem seed oil in a dermatological preparation targeted to be applied topically.

Creams are semisolid emulsion systems with opaque appearances, as contrasted with translucent ointments. Their consistency and rheologic character depend on whether the emulsion is a water-in-oil or oil-in-water type and the nature of the solids in the internal phase (Benard and Lazarus, 1987). Concern for the physical and chemical integrity of topical systems is no different from that of other dosage forms. However, there are some unique and germane dimensions to stability associated with semisolid systems (Gordon, 1995). Some of the factors to be evaluated for semisolids are stability of the active ingredient(s), stability of the adjuvants, visual appearance, colour, odour, viscosity, extrudability, loss of water and other volatile vehicle components, phase distribution, particle size distribution of dispersed phases, pH, texture, particulate contamination, microbial contamination, etc.

The chemical integrities of drug, preservatives and other key adjuvants must be assessed as a function of time to establish a product's useful shelf life from the chemical standpoint. Semisolid systems provide us with two special problems (Gordon, 1995). First semisolids are chemically complex, to the point that just separating drug and adjuvants from all other components is an analyst's nightmare. Many components interfere with standard assays, and, therefore difficult separations are the rule before anything can be analysed. Also, since semisolids undergo phase changes on heating, one cannot use high-temperature kinetics for stability prediction. Thus, stability has to be evaluated at the storage temperature of the formulation, and this takes a long time. Under those circumstances, problematic stability may not be evident until studies have been in progress for a year or more (Gordon, 1995). In the present work neem seed oil cream formulated using vanishing cream base is being evaluated physico-chemically over a period of twelve months

Materials and Method

Plant Species: - Ripe seeds of neem plant were collected from Osogbo, a town in South Western part of Nigeria. The plant specie from which the seeds were collected was authenticated at Forest Research Institute of Nigeria (FRIN) with FHL No. 107818. The seeds were sun dried until the moisture content was reduced to barest minimum. The seed kernels were later separated from the seed coat and stored in air tight containers.

Extraction of the Oil: - The dried seed kernels were communited using blender model MX-738 (Nakai, Japan). The blended materials were stored in air tight containers. Using the method of (Charmaine *et al*,2005). Normal hexane was used as solvent for extraction at seed weight: solvent ratio 1:10. The seeds were allowed to soak in the solvent for 8 days at room temperature. The solvent was then filtered through a whatman filter paper (No. 1) to remove the coarse seed materials, into pre-weighed sterile containers. The containers were covered with filter paper and the solvent was allowed to evaporate. The weight of the residue was calculated (Weight of the container plus extract minus the weight of the empty container) and the extracts were kept at room temperature.

Materials

500g of five different batches each containing stearic acid (1.0.1 Group, Malaysia) 13%$^{w}/_{w}$, Glyceryl monostearate (Croda Chemicals Ltd., England) 1%$^{w}/_{w}$, Glycerin (Oleo-Chemm Int., Singapore) 10%$^{w}/_{w}$, Potassium hydroxide (Allied Chemicals Ltd., UK) 0.9%$^{w}/_{w}$, Methyl parabens (Clariant, UK) 0.1%$^{w}/_{w}$, Propyl parabens (Clariant, UK) 0.05%$^{w}/_{w}$, five different concentrations range of neem seed oil i.e. 0%$^{w}/_{w}$, 2.5%$^{w}/_{w}$, 5.0%$^{w}/_{w}$, 7.5%$^{w}/_{w}$ and 10.0%$^{w}/_{w}$ for each batch respectively with 0%$^{w}/_{w}$ serving as control. Purified water was added to 100%$^{w}/_{w}$ in each case.

Preparation of the Cream

For each batch, 65g of stearic acid was accurately weighed into a beaker, 5g each of glyceryl monostearate and cetyl alcohol were added into the beaker. The mixture was heated until melted at about 60^{0}C. Then different concentrations of neem seed oil were incorporated. This was stirred together using AM-3250B magnetic stirrer (Surgefriend® Brand). This constitutes oilly phase. For the aqueous phase, 50g of glycerin, 4.5g of potassium hydroxide and purified water (Adequate to make 500g of cream) were separately weighed into a beaker and then heated until about 65^0C to aid dissolution of potassium hydroxide. To the aqueous phase was added the oilly phase slowly and stirred together with magnetic stirrer until crude emulsion was formed. This was cooled to about 50^0C and then homogenized with Silverson stirrer PA 0058 (homogenizer). This was later cooled with agitation until it congealed. The cream was later packaged into sterilized brown cream jars (20g each).

Physical Evaluation of Neem Seed Oil Cream:- This was carried out by observing the colour, odour, texture, feel, ease of administration, ease of removal, consistency, globule size at storage temperature for one year.

Rheology Evaluation:- Brookfield viscometer with spindle No. 2 and helipath attachment was used to determine the viscosity of the cream at different concentrations of neem seed oil once every month for a period of one year. This was done in triplicates at storage temperature of about 25^0C – 30^0C range.

pH Determination:- Determinations were made on 20g batches of the cream inside the jars by dipping the tip of Mettler Toledo pH meter (digital) inside the cream and then readings taken. This was carried out in triplicates on each batch once every month for a period of one year at temperature range of 25^0C – 30^0C.

Specific Gravity Determination:- This was carried out using a stainless disc with cover. The samples were packed into the disc tightly to the brim and the weights determined. The formula

$$\frac{\text{Weight of bottle + Content – Weight of bottle}}{8.2626}$$

was then used to calculate the specific gravity. This determination was carried out in triplicates.

Statistical Analysis: - One-way ANOVA was used to determine if there was significant difference in the pH, viscosity and specific gravity of the samples over a period of one year across the concentration range of the formulation. Also, student's t-Test (paired) was used to establish if there was significant difference in the mean values of the parameters studied by comparing values obtained at the commencement of the experiment i.e. 1st month and at the terminal end i.e. 12th month.

Results and Discussion

Extraction Yield: - The calculated yield is between (19 – 25% $^{w}/_{w}$) of dried seed kernel.

The neem seed kernel is an acrid bitter greenish yellow to brown oil. The calculated yield is between (19 – 25% $^{w}/_{w}$). The results of the physical evaluation of neem seed oil cream formulations over a period of one year is as presented in Table 1. Also chemical evaluation using pH, viscosity and specific gravity as parameters studied for a period of one year is as presented in Table 2 and graphically represented in Figs 1-3

Physical evaluation result shows that all the formulations (0% $^{w}/_{w}$ – 10.0% $^{w}/_{w}$ neem seed oil cream) retain their integrities throughout the entire period of study. There was no development of an off odour. The garlic odour observed in formulations 2.5% $^{w}/_{w}$ – 10.0% $^{w}/_{w}$ neem seed oil cream is characteristic of neem oil itself (Charmaine *et. al.*, 2005). Extensive oxidation of natural fatty materials (rancidification) is usually accompanied by development of a disagreeable odour (Jones, 1994). There was no change in the colour of the cream formulations under review. This implies that oxidation of fatty materials in the varnishing base was almost non existent perhaps due to the mode of packaging of the neem cream samples which was in brown cream jars with wax-sealed covers that was able to exclude oxidative catalytic factors such as air and light

The sample formulations also retained its whitish, homogenous soft textured appearance throughout the entire period of study. Changes in phase and texture are reasons for a suspect product sometimes. All the sample formulations were easy to apply and removable from the skin with both cold and warm water more so when the varnishing base is of $^{o}/_{w}$ emulsion type.

The particle size remains constant for all the formulations (0% $^{w}/_{w}$ – 10.0% $^{w}/_{w}$) throughout the entire period of study. Changes in particle size could be a consequence of crystal growth, changes in crystalline habit or the reversion of the crystalline materials to a more stable polymorphic form. Any crystalline alteration can lead to a pronounced reduction in the drug delivery capabilities and therapeutic usefulness of a formulation. Thus, products exhibiting such changes are seriously physically unstable and unusable.

Storage temperature fluctuating between 25^0C to 30^0C did not have adverse effect on the physical integrity of the homogenous state of the formulations during the study. It is a known fact that semisolid formulations undergo phase changes when exposed to high temperature and the more reason why high-temperature kinetics for stability prediction could not be employed for this study.

The pH of the formulations 0% $^w/_w$ to 10.0% $^w/_w$ is as presented in Table 1 and graphically represented in Fig 1. As can be seen, there was no significant change in the pH for the 12 months period of study even when tested at 5% significance level using student's t-test (paired,two-tailed) tool application for the data at first month of determination and 12^{th} month records. The probability of the significance of difference in means '*p*' was found to be greater than 0.05 i.e. ($p > 0.05$) leading to the fact that the data available seems consistent with null hypothesis of no significant difference. Changes in product pH is a sign of chemical decomposition, most probably of a hydrolytic nature, and if somehow detected are reason to return a product. In an earlier study ,Jarvis *et al* had reported that Azadirachtin,the major constituent of neem oil is most stable in mildly acidic aqueous solutions between pH 4 and 6 at room temperature. It is unstable in mildly alkaline and strongly acidic solutions. The pH of the cream base for the formulation of neem oil as can be seen in Table 2 and Fig1 i.e 0%w/w(control formulation) falls between pH 6 and 7.This is observed to be a favourable pH for the formulation of neem oil.The pH of all other formulations generally from Table2 falls around pH 6 and 7 showing no degradation of any kind

Time variable rheological behaviour of a semisolid may also signal physical or chemical changes. The viscosities of the formulations of neem seed oil cream (0% $^w/_w$ – 10.0% $^w/_w$) is presented in Table 2 and graphically represented in Figures 2. As can be seen, there was no significant difference across the 12 months of study for each of the formulations. Testing the significance of difference in means at 5% level indicates that ($p > 0.05$) pointing to the fact the data available appears consistent with null hypothesis of no significant difference across the months of study. Viscosity changes may signal Sometimes phase changes whereby there is slow rearrangement and contraction of internal structure. Eventually, here and there, globules of what is often clear liquid internal phase are squeezed out of the matrix. The main concern with a system that has undergone such separation is that a patient will not be applying a medium of uniform composition, because of unequal concentration of the drug relative to the other.

Also, changes in rheological behaviour could signal in stiffening of the semisolid formulations which may be as a result of oxidation (rancidification) of some fatty materials which could lead to discoloration and development of a disagreeable odour. If this occurs, it indicates compromise in chemical integrity of the product and is enough reason to return a product.

However, the viscosity of the formulations was of increasing order across the concentration range of 0% $^{w}/_{w}$ – 10.0% $^{w}/_{w}$. One-way ANOVA analysis at 5% test of significance of difference in means over a period of twelve months indicates that ($p < 0.05$) indicating that the difference is statistically significant since the data appears not consistent with null hypothesis of no significant difference. Increase in viscosity across the concentration range is probably as a result of increase in concentration of neem oil which itself is a fixed oil.

Generally, it appears that the specific gravity of formulations 2.5%$^{w}/_{w}$ – 10.0%$^{w}/_{w}$ from Table 2 and Fig 3 appear a little higher than that of 0%w/w over the 12 months period of study. This is probably due to absence of neem oil in the control formulation (0%$^{w}/_{w}$). Stiffening or liquefaction of formulations during storage are reasons that could lead to fluctuations in the specific gravity. These problems are absent in the formulations under review. This is why it appears that for all formulations 0%$^{w}/_{w}$ – 10.0%$^{w}/_{w}$, the specific gravity appears consistent across the months of study for each of the formulations.

Conclusion

The result of the investigations carried out so far had indicated stability for all parameters studied physically and chemically. For the whole period of 12 months, the formulations did not betray the design mechanism employed suggesting reliability at least for one year. In predicting the shelf life of the formulations of neem oil cream, one may be recommending at least one year shelf life which is a reasonable one for semisolid preparation. It also stands to reason that there was no breakdown of active ingredients in the formulations in this case, *Azadirachtin*, otherwise alteration in the pH of the formulations under study would have being experienced.

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Table 1: The Results of the Physical Evaluation of Neem Seed Oil Cream

Parameters	Samples				
	0%$^w/_w$	2.5%$^w/_w$	5.0%$^w/_w$	7.5%$^w/_w$	10.0%$^w/_w$
Appearance	Homogenous cream	Homogenous cream	√	√	√
Odour	Characteristic odour	Garlic odour	√	√	√
Texture	Soft	Soft	Very soft	Very soft	Very soft
Feel on skin	Slightly rough	smooth	Smooth	smooth and very light	Smooth and very light
Ease of application	Apply easily by massaging	√	√	√	√
Ease of removal	Easy to remove with cold water and warm water	√	√	√	√
Occlusiveness	Non-occlusive	√	√	√	√
Temperature effect (25^{0}C – 30^{0}C)	Stable	Stable	√	√	√
Particle size: Before homogenization	.15-o.51	.15-o.51	.15-o.51	.15-o.51	.15-o.51
After homogenization	.o8-o.21	.o8-o.21	.o8-o.21	.o8-o.21	.o8-o.21
Consistency: 1st – 12th month	Consistent	√	√	√	√

Table 2: Mean Viscosity, pH and Specific Gravity Determination for a Period of 12 Months

Concentrations		1st month	2nd month	3rd month	4th month	5th month	6th month	7th month	8th month	9th month	10th month	11th month	12th month
0.0%w/w	pH	7.07	6.77	6.82	6.84	6.86	6.86	6.86	6.94	6.88	7.06	6.43	6.97
	Viscosity (Poise)	950	850	850	950	960	950	950	850	900	950	950	900
	Specific gravity	0.99	0.98	1.01	1.00	0.99	0.99	0.98	1.00	1.00	0.99	1.00	1.00
2.5%$^{w}/_{w}$	pH	7.07	6.91	6.91	6.83	6.85	6.88	6.88	7.01	6.88	7.21	7.14	7.21
	Viscosity (Poise)	1000	950	950	970	800	800	800	950	900	850	970	900
	Specific gravity	1.01	1.01	1.06	1.01	1.00	1.06	1.02	1.01	1.03	1.01	1.01	1.02
5.0%$^{w}/_{w}$	pH	7.07	6.87	6.79	6.87	6.89	6.93	6.93	7.12	6.97	7.17	7.12	6.97
	Viscosity (Poise)	1300	1150	1500	1650	1200	1350	1350	1350	1300	1400	1350	1300
	Specific gravity	1.03	1.01	1.01	1.02	1.03	1.06	1.00	1.01	1.00	1.02	1.01	1.03
7.5%$^{w}/_{w}$	pH	7.20	6.90	6.96	6.95	6.93	6.92	6.92	7.00	6.97	7.00	6.98	6.98
	Viscosity (Poise)	1400	1750	1600	1700	1300	1500	1400	1500	1400	1500	1450	1500
	Specific gravity	1.02	1.02	1.02	1.01	1.02	1.00	1.01	1.02	1.02	1.01	1.02	1.02
10.0%$^{w}/_{w}$	pH	6.58	6.90	6.89	6.97	6.99	6.98	6.98	7.14	7.03	7.23	7.00	7.12
	Viscosity (Poise)	1900	2650	2900	3200	2600	2600	1750	2250	1700	1850	1900	2150
	Specific gravity	0.99	1.00	1.00	1.00	1.00	1.02	0.99	1.00	1.00	1.01	1.00	1.00

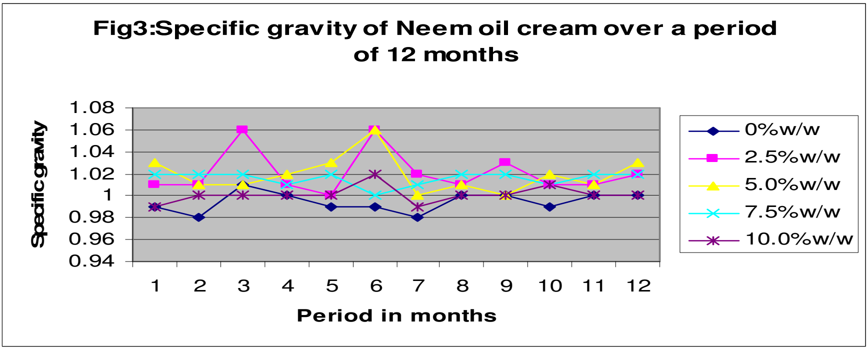
Fig3:Specific gravity of Neem oil cream over a period of 12 months
Specific gravity
1.08
1.06
1.04
1.02
1
0.98
0.96
0.94
1 2 3 4 5 6 7 8 9 10 11 12
Period in months
0%w/w
2.5%w/w
5.0%w/w
7.5%w/w
10.0%w/w