Int J Pharm Bio Sci 2013 Oct; 4(4): (P) 14 - 21

Research Article **Pharmaceutics**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

COMPARISON OF PHYSICAL CHARACTERISTICS OF VANISHING CREAM BASE, COW GHEE AND SHATA-DHAUTA-GHRITA AS PER PHARMACOPOEIAL STANDARDS

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ABSTRACT

Modern formulation science requires all excipients to be inert and to conform to Pharmacopoeial standards. However, Ayurveda, the ancient science of health from India believes that all substances possess therapeutic actions. Modern formulators have a choice of choosing from the modern formulation bases (e.g.; vanishing cream base) or Ayurvedic bases (e.g.; cow ghee) while formulating an herbal product. Nonetheless, all such bases must conform to specifications of physico-chemical properties that have the potential to affect the efficacy/ safety/stability of the product. In this study, we have compared the physical characteristics of a modern base (vanishing cream) with two Ayurvedic bases (clarified butter fat -cow ghee and Shata-dhauta-ghrita). Shata-dhauta-ghrita (SDG) is prepared by washing cow ghee 100 times with water and is prescribed for treatment of wounds, burns etc. All three bases were evaluated for their pH, viscosity, spreadability, organoleptic properties, globule size, acid value, saponification value, peroxide value, ester value, iodine value and free fatty acids. It was found that while cow ghee and vanishing cream base had comparable characteristics, SDG exhibits a much less degree of unsaturation (suggesting better physico-chemical stability) and better consistency (and hence suitability for topical applications). The paper also suggests the possible mechanism for improvement of these characteristics in the process of conversion of cow ghee to SDG. It further suggests that SDG might be a potential candidate as a base for topical preparations, esp. for wounds and burns.

KEYWORDS: **Shata dhauta ghrita, Cow ghee, Vanishing cream**

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INTRODUCTION

The traditional systems of medicine, evolved over centuries had been responsible for safeguarding healthcare of the world until the advent of allopathic system of medicine. As the latter system used knowledge of modern biology and chemistry, for both discovery and treatment, it found fast acceptability among the users and now it occupies predominant space in the area of healthcare. In spite of this, the contribution of the traditional preparations, which are normally polyherbal, is increasing because of the general impression that these products are safe; while the single-molecule based modern drugs used in allopathic system can have severe adverse effects. [1] Several modern cream bases are available for preparation of topical formulations. Their constituents like beeswax, stearic acid, liquid paraffin are characterized by their inertness, as they do not have any therapeutic activity. However, an Ayurvedic base like Cow ghee is reported to possess various activities/properties. Caraka clearly states the indications for ghee: "(it) promotes memory, intelligence, agni (factor responsible for digestion, metabolism, and biotransformation), semen, ojas (bio-essence of life), Kapha (one of the three bio-energies mainly responsible for cohesiveness) and medas (adipose tissue). It alleviates Vata (one of the three bio-energies mainly responsible for movement), Pitta (one of the three bio-energies mainly responsible for heat), poison, insanity, phthisis, inauspiciousness and fever. It is the best of all fats, is cold, madhura rasa, madhura vipaka, has 1000 potentialities and so, if used properly according to prescribed methods, exerts 1000 types of actions.[2] If it is used as a base for preparation of cream formulations, the formulation shows synergistic effect with the active ingredients. Bramhi ghrita a polyherbal Ayurvedic formulation is recommended in the management of psychological disorders like Unmad (insanity), Apasmara (epilepsy) and Graharogas (idiopathic psychological disorders).[3] Hingusauvarchaladi Ghrita and Saptarvartita Hingusauvarchaladi Ghrita showed anticonvulsant activity.[4] Manjishthadi Ghrita can be prescribed as a local healing agent for common wound. [5] Wound healing activity is also shown by Darvhi Ghrita. [6] Anti-inflammatory activity by Jatyadi ghrita, etc. [7] Sedative and anticonvulsant activities of Unmadnashak Ghrita.[8] Shata-dhauta-ghrita (SDG) is a 100 times washed (Shata = one hundred, dhauta = washed) ghee, i.e. clarified butterfat. It is mentioned in traditional texts for the management of conditions like burns, chicken pox, leprosy, wounds, and other skin diseases and as a vehicle for drugs to be applied externally. [9-10] In this study, cow ghee has been evaluated for its physicochemical parameters and the changes in these parameters during repeated washing ((resulting in the formation of SDG) have been recorded. Finally, the physicochemical properties of cow ghee and SDG have been compared with those of a modern vanishing cream base used in similar formulations. Hence, an attempt is being made through this paper to find out the rationale behind washing cow ghee 100 times with water. The aim of present study investigation is to prepare and evaluate Shata dhauta ghrita and compare it with modern vanishing cream base formulation and with cow ghee for its physicochemical parameters.

MATERIALS AND METHODS

Table 1 and Table 2 provide the list of instruments and materials used in the preparation of the formulation resp.

Table 1
List of instruments used in preparation of formulation

Instruments/Models	Purpose
Weight balance, Mettler XP26	API / excipients weighing
pH Meter, Eutech PH 2700	Measurement of pH
Brookfield Viscometer DV II + Pro	Determination of viscosity
Remi Centrifuge, Remi R-4C	Centrifugation of formulation
Mechanical Agitator, Salomix, SL	Uniform mixing/ agitation
Motic microscope, DM –B1	Globule size determination

Table 2
List of materials used in preparation of formulation

Material	Source
Cow ghee	Shridhar Pvt. Ltd
Liquid paraffin	Bendale Chemicals
Stearic acid	Loba Chemicals
Triethanolamine	Loba Chemicals
Carboxymethyl cellulose	Clariant (Nipasol)
Cety alcohol	Merck Ltd
Sodium alginate	Clariant (Nipasol)
Glycerine	Colorcon Asia Pvt. Ltd

METHODOLOGY

All chemicals and solvents used were of analytical grade. All the results were obtained by repetition of the each experiment at least three times. Cow ghee, SDG and modern cream base were observed for their organoleptic properties and analyzed for various physicochemical parameters prescribed for lipids in pharmacopoeias, e.g.; Viscosity, acid value, saponification value, iodine value, peroxide value etc. [11-18] Globule size of these three bases was also determined using Motic Microscope.

Preparation of Shata-dhauta-ghrita

The glass vessels were cleaned thoroughly and rinsed with distilled water. Previously standardized cow ghee (2.5 kg) was taken in a glass vessel. Distilled water (1.5 L) was added to it. With the help of mechanical agitator, the mixture of cow ghee and water was agitated for 5-8 minutes. The contents were allowed to settle. Water was decanted carefully, avoiding loss of ghee. The fresh slot of 1.5 L distilled water was added to the previously washed cow ghee and similar procedure was repeated. This operation was carried out one hundred times to obtain SDG. Samples were collected after washing and stored in plastic containers at room temperature for analysis. [19]

Preparation of Modern cream base (MCB)

The following three formulations of o/w cream base were prepared and evaluated based on their spreadability, physical stability and wash cream base property**.** Table 3 provides the composition of the three formulations.

Table 3
Formula for formulation of Modern cream bases

Ingredients	F1	F2	F3
Mineral oil	30	29	10
Stearic acid	10	13.5	3
Triethanolamine	2	1.8	1.8
Carboxymethyl cellulose	0.5	-	-
Water	57	52	85
Sodium alginate	-	1.8	-
Total quantity (gm)	100	100	100

Formulation F1 was selected based on the above-mentioned parameters.

Analysis of Organoleptic properties
Cow ghee, SDG and F1 were tested for color, odor, taste and texture.

Analysis of physical parameters
Determination of pH
Accurately weighed 5 g of the sample was dispersed in 45 ml. of water. The pH of the suspension was determined at 27°C using digital pH meter.

Determination of Viscosity
The viscosity determinations were carried out u sing a Brookfield Viscometer (DV II+ Pro model) using spindle number S-64 at a 20 rpm at a temperature of 25.1^0 C. The determinations were carried out in triplicate and the average of three readings was recorded.

Determination of Spreadability
Spread ability may be expressed by the extent of area to which the topical application spreads when applied to the affected parts on the skin. The therapeutic efficiency of the formulation also depends upon its spreading value. Hence, it was found necessary to determine the spread ability of the formulation. For this purpose, ample (about 3gm) was applied in between two glass slides and they were pressed together to obtain a film of uniform thickness by placing 1000 gm weight for 5 minutes. Thereafter a weight (10gm) was added to the pan and the top plate was subjected to pull with the help of string attached to the hook. The time in which the upper glass slide moves over the lower plate to cover a distance of 10 cm is noted. The spread ability (S) can be calculated using the formula.

$$S = m \times \frac{L}{T}$$

Where,
S – Spread ability
m- Weight tied to upper glass slide.
l- Length moved on a glass slide
t- Time taken.
The determinations were carried out in triplicate and the average of three readings was recorded.

Determination of globule size by Motic microscope
Visualization of the three bases was done by Compound Digital Biological Microscope (DM-B1 Motic microscope, Shanghai, China). The optical microscope was attached with the software Digipro V 4.0, through which image analysis was done, photographs were captured. A small amount of sample was placed on a glass slide, covered using cover slip and observed under microscope.

Determination of chemical parameters

Acid value, ester value, saponification value, iodine value, peroxide value of cow ghee, SDG and F1 were determined by the methods outlined in pharmacopoeias.[11-18]

RESULTS

Organoleptic properties

Cow ghee, SDG and modern cream base were observed for its organoleptic properties, the results of which are shown in Table 4.

Table 4
Determination of organoleptic properties

Organoleptic Property	Cow ghee	SDG	Modern cream base
Colour	Golden yellow	White	White
Odour	Pleasant	Odourless	Odourless
Taste	Characteristics	Tasteless	Tasteless
Texture	Granular, Oily	Smooth,Non oily	Smooth

Physical Parameters

The results of the assessment of various physical properties of cow ghee, SDG and modern cream base are shown in Tables 5-7, while their images captured on Motic microscope are shown in Fig. 1-3.

Table 5
Determination of pH and viscosity

Sr. No	Formulations	Ph	Viscosity, cp	Spreadability (g.cm/sec)
1	Cow ghee	6.33±0.41	8020 ±26.4	7.18±0.25
2	SDG	7.56±0.35	10279 ±26.1	9.65±0.15
3	Modern cream base	8.46±0.40	14068 ±18.5	9.39±0.33

All the values are express as Mean ± SD for n=3 readings

Table 6
Determination of Globule size

Formulations	SDG	Cow ghee	MCB
Maximum globule surface area $(\mu m)^2$	55.4 ±0.45	97.8±0.66	20.3±0.67
Maximum globule perimeter (µm)	71.4 ±0.45	153.2 ±0.65	15.4±0.30
Average Globule Size (µm)	3.4 ±0.30	5.2 ±0.47	2.3±0.45

All the values are express as Mean ± SD for n=3 readings

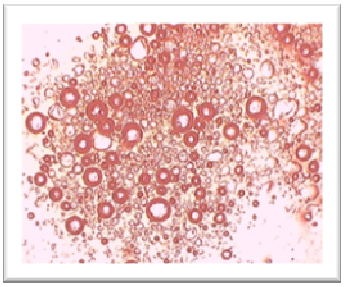


Image 1
Cow ghee

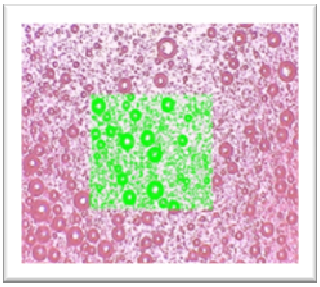


Image 2
SDG

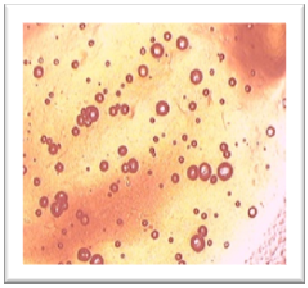


Image 3
Modern cream base

Chemical parameters

Cow ghee, SDG and Modern cream base were compared for their chemical parameters, the results of which are shown in Table 7.

Table 7
Comparison of Chemical Parameters

Chemical Parameters	Cow ghee	SDG	MCB
Acid value	1.79±0.01	0.89±0.01	1.64±0.02
Ester value	247.6±0.37	139.5±0.41	122.6±0.3
Saponification value	249.4±0.32	140.5±0.47	124.3±0.41
Iodine value	35.49±0.35	2.82±0.05	55.4±0.45
Peroxide value	1.63±0.25	1.36±0.15	1.65±0.05

All the values are express as Mean ± SD for n=3 readings

Fatty acid composition

Cow ghee, SDG and modern cream base were evaluated based on their fatty acid composition, the results of which are shown in Table 8.

Table 8
Composition of fatty acids

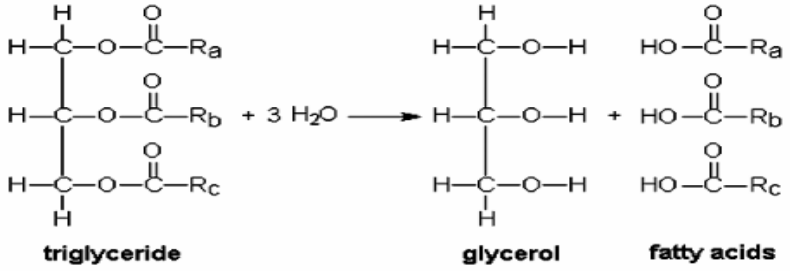
Free fatty acid	Cow ghee	SDG	MCB
Oleic acid	0.86±0.02	0.44±0.01	0.81±0.36
Palmitic acid	0.81±0.01	0.40±0.25	0.73±0.05
Lauric acid	0.63±0.04	0.31±0.15	0.57±0.01

All the values are express as Mean ± SD for n=3 readings

DISCUSSION

A comparison of physicochemical parameters of cow ghee, SDG and modern cream base has been done. Preparation of SDG can be illustrated as follows. Initially, the pure lipid phase, i.e. cow ghee comes in contact with an aqueous phase. Due to agitation, it results in formation of w/o type of emulsion as lipid phase (Cow Ghee) is a major phase. As the washing continuous, due to pressure applied during agitation, particle size of fat granules gets reduced. Eventually, successive washings result in o/w type of emulsion. It is possible that it might lead to formation of a complex system like w/o/w emulsion. The characteristic granular, oily consistency and odor present in the cow ghee is lost, resulting in a homogeneous, smooth, non oily product, which is easier to apply, thus improving the patient compliance as

a base for topical application. pH change from acidic to neutral makes it beneficial to prevent skin irritation. Hence, the preparation may be applied on open wounds. Reduction in particle size of SDG makes the product non- granular, non-sticky, homogeneous, with a large surface area, similar to that of modern cream base which makes it easy to apply on skin and may result in increased rate of absorption through skin. Viscosity of SDG was found to have increased in the conversion from cow ghee to SDG, with its value similar to that of modern cream base. Washing results in the formation of a homogenous mass of oil in water emulsion with better consistency and viscosity which helps in its topical application and is beneficial in removal of scars and as anti inflammatory activity. Decrease in iodine value of SDG indicates the decrease in degree of unsaturation, which eventually reduces the chances of rancidity, thus increase stability of the product. Iodine value of cow ghee and modern cream base are similar and much higher than that of SDG, thus indicating the higher degree of unsaturation in these bases. Decrease in acid value with repeated washings indicates the reduction in free fatty acids. Here too, cow ghee and modern cream show much higher values (and hence susceptibility to rancidity) as compared to SDG. Our findings are consistent with the following mechanism of formation of SDG, which is also supported in literature. [20] Fat splitting is a process in which, fat is hydrolyzed in the presence of water to yield free fatty acids and glycerols. High temperature and pressure are known to accelerate the process of hydrolysis. In the preparation of SDG, although temperature is kept constant, there is repeated and prolonged washing of the ghee and fat mixture. Thus the pressure factor may contribute to fat splitting. After each washing, aqueous phase is withdrawn and replaced by fresh slot of the same. If the reactants and products are not removed from the sphere of the reaction, equilibrium will eventually be reached, depending upon the concentrations of the former. In practice, by using a large excess of water and repeatedly withdrawing the glycerol rich aqueous phase and replacing it with fresh water, the process of fat splitting is accelerated. Decrease in the degree of unsaturation can be supported by the absence of unsaturated fatty acids such as oleic acid, palmitic acid and lauric acid in SDG. Cow ghee has been reported to form a complex with diclofenac sodium[21]. It would be interesting to find whether SDG is free from such complexation. Moreover, it would be clinically important to note whether the altered nature of fatty acids in SDG make it a better wound healing agent in wounds an burns than cow ghee.



From the present work, it can be concluded that changes taking place in cow ghee while washing it with 100 times to prepare Shata dhauta ghrita, makes it an elegant and suitable product for topical application. Hence, it may be used as a better alternative to cow ghee and modern cream base for the preparation of cream formulation, esp. for burns and wounds.

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