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"FORMULATION AND EVALUATION OF HERBAL ANTI-ACNE FACE WASH"

Jhansi Rani M.*[1], Akhil S.[2], Triveni T.[3], Revathi P.[4], Lakshmi Prasanna J.[5] and Dr. A. M. S. Sudhakar Babu[6]

*[1]Assistant Professor, Department of Pharmaceutics, A.M Reddy Memorial College of Pharmacy, Narasaraopet, Guntur (Dt), India.
[2,3,4]Student, A.M Reddy Memorial College of Pharmacy, Narasaraopet.
[5]Assistant Professor, Department of Pharmaceutics, A.M Reddy Memorial College of Pharmacy, Narasaraopet, Guntur(dt), India.
[6]Principal, A.M Reddy Memorial College of Pharmacy, Narasaraopet, Guntur (Dt), India.

***Corresponding Author: Jhansi Rani M.**
Assistant Professor, Department of Pharmaceutics, A.M Reddy Memorial College of Pharmacy, Narasaraopet, Guntur (Dt), India.

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ABSTRACT
Acne is an inflammatory disease of sebaceous follicles of skin. Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Herbal formulations have growing demand in the market. The present work deals with the development and evaluation of the herbal anti-acne face wash containing aqueous extracts of roots of burdock, liquorice, shahi jeera, orange peel and fruit of nut meg. Although various topical herbal formulations available in the market, we propose to make pure herbal formulation without any synthetic ingredient. The plants have been reported in literature having good anti-microbial, anti-oxidant and anti-inflammatory properties. Various formulation batches i.e., F1 to F5 were prepared using Xanthan gum in varied concentrations. Prepared formulations were evaluated for various physical parameters like colour, appearance, washability, pH, Spreadability and anti-microbial activity. Optimised batch of formulation compared with marketed formulation. Amongst all the formulation studies F2 was found optimum for all the parameters.

KEY WORDS: Acne vulgaris, Burdock root, Anti-microbial activity.

INTRODUCTION
Acne vulgaris is a common skin disorder affecting more than 85%population of the world, specifically teenagers and adolescents. The term acne is derived from Greek word "acme" which means prime of life. Although generally considered to be a benign, self- limiting condition, acne may cause severe psychological problems or disfiguring scars that can persist for a long time. It is a pleomorphic disorder and can manifest at any time during life but it most commonly present between age of 12-24, which estimates 85% of population affected. Nearly 90% of teenagers have acne and half of them continue to experience symptoms as adults.[1,2,3] By age 40 years 1% of men and 5% of women still have lesions.[4] Although acne is not a life threatening condition, it can have detrimental effects on the quality of life of affected individuals. A recent analysis shows an prevalence of acne in children because of pubertal onset.[5]

CLINICAL CHARECTERESTICS OF ACNE
A spectrum of lesions may be present, including non-inflammatory open and closed comedones (black heads and white heads respectively) and inflammatory papules, pustules, nodules and cysts. Lesions may be present on the face, neck, chest or back-areas with the greater density of pilosebaceous units.[6] Comedone formation is intrinsic to the diagnosis of acne vulgaris-when not clinically apparent, consider alternative diagnosis.

Several groups have proposed standardized measures for classifying acne, although none has been universally accepted.[7,8] Classification is important because it helps to inform treatment strategies.[9]

Acne is categorized broadly into three types. They are
MILD ACNE
Mild acne is typically limited to the face and it is charecterised by the presence of non inflammatory closed and open comedones with few inflammatory lesions.

MODERATE ACNE
Moderate acne is charecterised by an increased number of inflammatory papules and pustules on the face and often mild truncal disease.

SEVERE ACNE: Finally acne is considered to be severe when nodules and cysts are present. In these cases

facial lesions are often accompanied by widespread truncal disease.

PATHOGENESIS OF ACNE VULGARIS

Acne is a disease of pilosebaceous glands. Multiple factors are responsible for pathogenesis of acne. They are

- Altered follicular keratinization.
- Sebum.
- Harmones.
- Bacterial colonization.
- Inflammation.
- Nutrition

Altered follicular keratinisation: It marks the most primary change in the pilosebaceous unit in acne patients. Desquamated cornified cells of upper canal of the follicle become abnormally adherent; instead of undergoing the normal process of shedding and discharge through follicular orifice, these cells form a retained, microscopic hyperkeratotic plug (the microcomedo) in follicular canal. This process called comedogenesis. The progressive enlargement of microcomedo give rise to clinically visible comedo which can be open comedo/ black heads (appearing flat or slightly raised and distend from follicular orifice), have black colour due to oxidation of melanin pigment, or as closed comedo / white heads, having closed overlying surface.[10]

Sebum: Sebum is the lipid- rich secretion product of sebaceous gland, secreted by sebocytes which along with keratinocytes may act as immune cells of the skin. The severity of acne is directly proportional to the sebum production.[11] The secretion of sebum increases due to enlargement of sebaceous glands under the stimulatory action of androgens. The sebum of acne patients is characterized by the presence of lipoperoxides due to peroxidation of squalene and diminished levels of sebum antioxidant vitamin E.

Hormones: Androgens have only the priming role in acne development as they (testosterone and dehyrotestosterone) stimulate proliferation and differentiation of sebocytes and infundibular keratinocytes.[12] During puberty, increased dehyrotestosterone (DHT) may lead to hyperkeratinisation by their action on infundibular keratinocytes. The hyperkeratinisation in follicular infundibulum and sebaceous duct is one of the most crucial events in the development of acne lesions.

Bacterial colonization: Propionibacterium acne is an anaerobic obligate diptheroid that resides beneath the surface of human skin and populates the androgen stimulated sebaceous follicles. The oxidative stress within the pilosebaceous unit changes the environment from aerobic to anaerobic which is the best suited for this gram positive bacterium. It causes inflammatory acne.[13] Staphylococcus epidermidis is also the resident of human skin flora and is the aerobic organism associated with superficial infections within the sebaceous units.

Inflammation: Inflammation is the direct or indirect result of proliferation of P. acne. The bacteria produce the extracellular lipase that hydrolyses sebum triglycerides to glycerol, used by organism as growth substrate, and fatty acids, which have proinflammatory and comedogenic properties. Further, P.acne may activate keratinocytes and sebocytes via TLR, CD14 and CD1 molecules. TLR2 is expressed on the surface of macrophages surrounding the pilosebaceous follicles in acne lesions. Activation of TLR2 leads to triggering of transcription factor nuclear factor and thus the production of cytokines which along with IL8 and IL 12, released from TLR2 positive monocytes, produce the inflammatory lesions of acne. Inflammatory acne comprises of pustules, papules and nodules.[12]

Nutrition: Acne is also driven by growth factors (particularly insulin-like growth factor [IGF-1]) acting on sebaceous glands and keratinocytes lining the pilary canal. Dairy products contain 5α-reduced steroid hormones and other steroid precursors of DHT that drive sebaceous gland function. Drinking milk causes a direct rise in IGF-1 through a disproportionate elevation in blood sugar and serum insulin level. High glycemic load foods also cause IGF-1 mediated elevations in DHT. IGF-1 levels during teenage years closely parallel acne activity and are likely synergistic with the steroid harmones.[14]

Due to increased instances of resistance of acne inducing bacteria towards the antibiotics18, the alternate system of medicine for the treatment of acne have been investigated and adopted. Among the alternate systems of medicine the topical therapeutic agents are more convenient for application. The herbs as ingredients in topical acne treatment are occupying the upper position as they are safe, dilute, patient familiar, economic, easily available and multifunctional.[15] Figures from the World Health Organization suggest that 4 billion people, who make nearly 70% of the world's population, are users of herbal medicine for some purpose of primary health care. Medicinal herbs have always been in usage in some form or the other in indigenous systems of medicines including Ayurveda, Sidha and Unani in India.

Among those herbs it is prove to have anti-bacterial and anti-inflammatory properties. In ayurveda burdock is considered as an alternative and cleaning herb with microbial and anti inflammatory properties that may be beneficial for acne. Preliminary research confirms that burdock has antioxidant, anti inflammatory and anti bacterial properties and suggests that it may also have hormonal effects. Controlled animal studies have shown that burdock extracts reverse hyperproliferation of skin cells a symptom of both psoriasis and acne. The linoleic acid content of burdock is believed to be responsible for its inhibitory effects against hyperproliferation.[16]

MATERIALS AND METHODS

MATERIALS

LIST OF INGREDIENTS

Table-1 list of ingredients

S. No	Ingredients	Uses
1.	Burdock root (Arctium Lappa)	Anti bacterial, Anti inflammatory and highly beneficial for acne prone skin.
2.	Nutmeg seed (Myristica fragrance)	Antibacterial, antifungal, antiseptic, bactericide.
3.	Liquorice root (Glycyrrhiza glabra)	Delivers valuable soothing properties to the skin. Highly rejuvenating an nutritive qualities are attributed to it.
4.	Honey	Light humectants and nutrient used as a thickening agent to give body to facial creams, creams and lotions.
5.	Shahi jeera	As perfume
6.	Lemon juice	To lighten skin and reduce blemish marks on the skin. It also quite effective for treating acne and pimples and as a natural pH adjuster in cosmetics.
7.	Xanthan Gum	A gum produced by the pure culture fermentation of a carbohydrate also called corn sugar gum. It is used as a non toxic thickener and stabilizer.
8.	Orange peel extract	Anti-oxidant, anti-inflammatory, anti-microbial, Orange peel properties can maintain the natural balance of skin oils and tighten the skin by absorbing excess oils and removing dead skin cells.
9.	Rose water	Used as solvent; it also has antibacterial and antiseptic properties which eventually cures acne.
10.	Walnut	Scrubbing action of walnut granules helps break up the mild oil deposits and clear away dead skin cells and debris.
11.	Sodium lauryl sulphate	Used as a foaming agent.

METHODS

COLLECTION

Root of burdock was bought in online at EBAY.IN. Fruits of Nutmeg, Shahi jeera, orange peel, liquorice root, rose water were collected from the local market of narasaraopet.

Preparation of extracts: Roots of burdock, roots of liquorice, orange peel were kept in hot air oven for drying purpose at 45^0c and grinded into small pieces by using grinder. Seeds of nutmeg and shahi jeera (cumin) were crushed to make powder.

Prepared powders were extracted by dynamic maceration with rose water in 1:5 ratio at room temperature for three days and then they are filtered.

Extraction process of burdock root by maceration process: Fresh milled roots were successively extracted by dynamic maceration with rose water (1:5 plant/solvent ratio, 3 times each solvent), at room temperature for three days then it is filtered.

Filteration: Filtration of extract was done by using simple filter paper and funnel for two times.

Evaporation: Evaporation was done by using electronic water bath. Filtrate were allowed to evaporate in evaporating pan at 60^0c temperature until the desired concentration of the extract was obtained.

Development of Formulation: Various formulation batches were prepared according to the table. The desired concentration of gelling agent i.e. xanthan gum was weighed accurately and dispersed in hot rose water(not more than 60^0c; 50% weight of the batch size) with moderate stirring, avoiding air entrapment and allowed to soak overnight. Desired quantity of lemon juice was dissolved in desired quantity of honey by gentle stirring. Desired quantity of concentrated herbal extracts were added to the remaining amount of rose water and mixed with above honey mixture by gentle stirring. This was finally mixed with previously soaked gel formulation. Sodium lauryl sulphate is used as a foaming agent. Prepared formulations were filled in suitable container and labeled accordingly.

Table-2: Various formulations of extracts

S.no	Ingredients	Quantity taken for 10g gel F1	F2	F3	F4	F5
1.	Burdock root extract	0.7ml	0.75ml	1.0ml	0.7ml	0.7ml
2.	Nutmeg extract	0.5ml	0.25ml	0.5ml	0.25ml	0.25ml
3.	Orange peel extract	0.1ml	0.1ml	0.1ml	0.1ml	0.1ml

4.	Liquorice extract	0.25ml	0.25ml	0.25ml	0.25ml	0.25ml
5.	Shahi jeera extract	0.1ml	0.1ml	0.1ml	0.1ml	0.1ml
6.	Lemon juice extract	0.1ml	0.1ml	0.1ml	0.1ml	0.1ml
7.	Honey	0.5ml	0.5ml	0.5ml	0.5ml	0.5ml
8.	Xanthan gum	0.05g	0.1g	0.15g	0.02g	0.01g
9.	Exfoliating Walnut granules	q.s	q.s	q.s	q.s	q.s
10.	Rose water	q.s	q.s	q.s	q.s	q.s
11.	Sodium Lauryl sulphate	q.s	q.s	q.s	q.s	q.s

EVALUATION OF FORMULATION

Physical evaluation tests

Physical parameters such as colour, appearance & consistency were checked visually.

1. Washability

Formulations were applied on the skin & then ease & extent of washing with water were checked manually.

2. pH

pH of 1% aqueous solution of the formulation was measured by using a calibrated digital pH meter at constant temperature.[17]

3. Spreadability

Spreadability denotes the extent of area to which the gel readily spread on application to skin or the affected part. The bioavailability efficiency of a gel formulation also depends on its spreading value[18]. The spreadability is expressed in terms of time in seconds taken by two slides to slip off from the gel, placed in between the slides, under certain load. Lesser the time taken for separation of two slides, better the spreadability. Two sets of glass slides of standard dimensions were taken. The herbal gel formulation was placed over one of the slides. The other slide was placed on the top of the gel, such that the gel was sandwich between the two slides in an area occupied by a distance of 6 cm along the slide. 100gm weight was placed upon the upper slide so that the gel between the two slides was pressed uniformly to from a thin layer. The weight was removed & the excess of the gel adhering to the slides was scrapped off. The two slides in position were fixed to stand without slightest disturbance & in such a way that only the upper slide to slip off freely by the force of weight tied to it. A 20gm weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 6 cm7 separated away from the lower slide under the influence of the weight was noted. The experiment was repeated three times both formulated gels & marketed gel & the mean time taken for calculation.[103 104]

Spreadability was calculated by using the following formula,

$S=M\times L/T$

Where,
S- Spreadability
M- Weight tied to the upper slide (20gm).
L- Length of the glass (6.5cm).
T- Time in sec.

DETERMINATION OF ANTI MICROBIAL ACTIVITY

Type of medium

ASLA Agar Base

ASLA Agar is used for selective isolation and cultivation of *Propionibacterium* species from foods.

Table-3 Composition of ASLA agar medium

S. No	Ingredients	Composition Gms/lit
1.	Ammonium sulphate	3.000
2.	Disodium phosphate	1.200
3.	Mono potassium phosphate	1.200
4.	Manganese sulphate	0.050
5.	Magnesium sulphate	0.200
6.	Ferric sulphate	0.040
7.	L-Cysteine hydrochloride	0.500
8.	Agar	10.000
9.	Final pH (at 25^0C)	6.5±0.2

Preparation of media: Suspend 8.1 grams in 500 ml distilled water. Heat it to dissolve the medium completely. Add 10 grams of Sodium lactate. Mix well and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45°C and aseptically add rehydrated contents of 1 vial of Propionibacteria Growth Supplement (FD097). Mix thoroughly and pour into sterile Petri plates or tubes.

Procedure: The anti bacterial activity was determined by Cup plate method. Propionibacterium acne (p.acne) was incubated in ASLA agar medium for 48 hours under anaerobic conditions and adjusted to yield approximately 1×10^8 CFU/ml. Each petri dish was filled to a depth of 4-5 mm with a ASLA agar medium that was previously inoculated with suitable inoculums of suitable test organism, and then allowed to solidify. The petri dishes

were sterilized in hot air oven for 30 min before use. Small sterile borer of uniform size was placed approximately at 10 cm height, having an internal diameter of approximately 6-8 mm and made of alluminium or stainless steel. Each plate was divided into 4 equal portions along the diameter. To each portion one cylindrical cavity was made in medium with the help of sterile borer. The petri dishes were incubated at 37^0c for 18 hrs. Diameter of the zone of inhibition was measured and the average diameter for each formulation was calculated by using antibiotic zone reader. The diameter obtained by the test sample was compared with that produced by the marketed formulation.

Composition of nutrient agar medium

Table-4 composition of nutrient agar medium

S. No	Ingredients	Quantity
1	Peptone	0.5%
2	Beef extract	0.3%
3	Agar	1.5%
4	Nacl	0.5%
5	Distilled water	Upto 1000ml

Preparation of nutrient agar medium: Suspend 28 gms of nutrient agar powder in one litre of distilled water. Heat this mixture with stirring to fully dissolve all components. Sterilize the dissolved mixture at 121^0c for 15min. Once the nutrient agar has been sterilized in an autoclave, allow it to cool but not solidify.

Procedure: The culture of staphylococcus epidermidis was prepared in nutrient agar medium at 24hrs for aerobic conditions. Each petri dish was filled to a depth of 4-5 mm with a nutrient agar medium that was previously inoculated with suitable inoculums of suitable test organism, and then allowed to solidify. The petri dishes were sterilized in hot air oven for 30 min before use. Small sterile borer of uniform size was placed approximately at 10 cm height, having an internal diameter of approximately 6-8 mm and made of alluminium or stainless steel. Each plate was divided into 4 equal portions along the diameter. To each portion one cylindrical cavity was made in medium with the help of sterile borer. The petri dishes were incubated at 37^0c for 18 hrs. Diameter of the zone of inhibition was measured and the average diameter for each formulation was calculated by using antibiotic zone reader. The diameter obtained by the test sample was compared with that produced by the marketed formulation.

RESULTS AND DISCUSSION

Evaluation of formulation

Physical parameters

Table-3: Evaluation of physical parameters

Formulation/ Batch code	Colour	Consistency	Washability	pH	Spreadabilty (gm-cm/sec)
Marketed	Green	Semi solid	Good	5.8	5.909
F1	Orange	Semi solid	Good	4.4	3.251
F2	Orange	Semi solid	Good	5.4	5.527
F3	Orange	Semi solid	Good	5.2	2.631
F4	Orange	Liquid	Good	4.7	4.654
F5	Orange	Liquid	Good	4.1	6.541

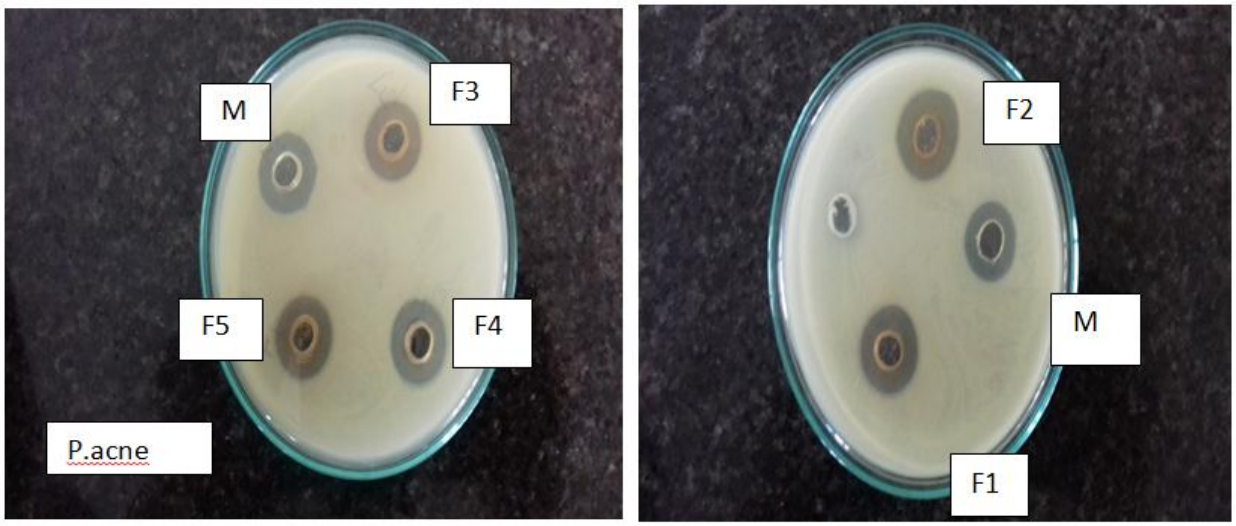


Fig-1 Representation of zone of inhibition for various formulations

Determination of anti microbial activity

Table-4: Evaluation of anti microbial activity

S. No	Standard and test samples	Propionibacterium acne	Staphylococcus epidermidis
1.	Himalaya neem face wash	11mm	10mm
2.	Formulation 1	12mm	11mm
3.	Formulation 11	15mm	13mm
4.	Formulation 111	14mm	12mm
5.	Formulation 1V	10mm	9mm
6.	Formulation V	8mm	6mm

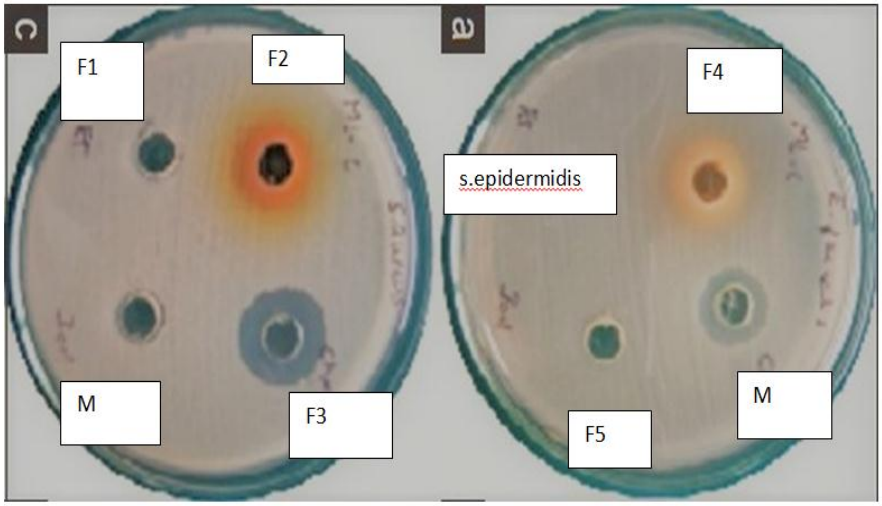
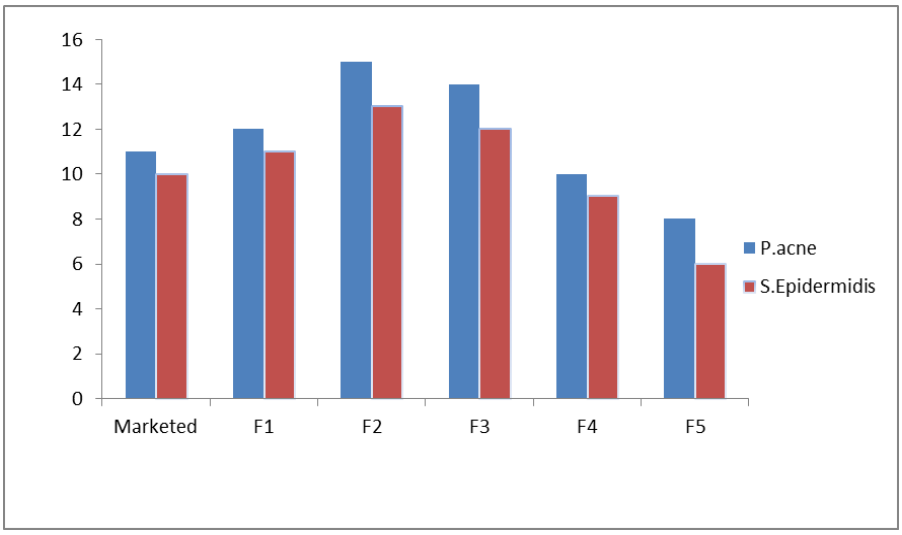


Fig-2 Representation of zone of inhibition for various formulations



SUMMARY AND CONCLUSION

Skin disease is very common and the need to prevent or treat the disease is in great demand. Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Herbal formulations have growing demand in the world market. It is a very good attempt to establish the herbal face wash containing aqueous extracts of burdock root, liquorice root and seeds of nutmeg and jeera. Different formulations were developed by varying the properties of anti-acne agents and thickening agents. For the developed preparations various physical evaluation tests like pH, color, Washability, consistency and spreadability were performed and anti microbial activity of the developed formulation was studied by cup plate method as the length of zone of inhibition were measured in mm and finally based on the evaluation tests we concluded that F2 formulation possess all the properties within the limit showing the anti microbial activity indicated by the length of zone of inhibition. F2 is better herbal face wash formulation of all the developed formulations.

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