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Ensuring Stability of *Panchavalkala*-Based Ayurvedic Wound Healing Gels: A Six-Month Accelerated Study

Dr. Griva Bhatt [1], Dr. Kunal Gohil [2], Bhumi Kantariya [3], Dr. Palak Vyas Trivedi[4]

[1,2]*Assistant Professor, Dept. of R. S. & B. K., ITRA-Pharmacy, Jamnagar, India*
[3]*Assistant Professor, Dept. of Pharmaceutical Chemistry, ITRA-Pharmacy, Jamnagar, India*
[4]*Head, R & D, Vivah Herbals and Healthcare, Ahmedabad, India*

Abstract*— *Background: **Stability assessment is essential for determining shelf life and guaranteeing the quality, safety, and efficacy of pharmaceutical products. Ayurvedic formulations modernized into contemporary dosage forms require comprehensive stability assessment following regulatory guidelines to meet international pharmaceutical standards.**

Objective: **The goal of this research work is to determine if two new Ayurvedic gel formulations, for healing wounds are stable. These are the N-Gel that has *Panchavalkala*, Nimba and Kumari in it and the H-Gel that has *Panchavalkala*, Haridra and Kumari in it.**

Methods: **To do this we followed the rules set by the Drugs and Cosmetics Act of India and the World Health Organization guidelines. We kept the gels in a room that was very hot and humid with a temperature of 40 degrees Celsius and humidity of 75 percent for six months. This is called storage and it helps us find out if the Ayurvedic wound healing gel formulations, specifically the N-Gel and the H-Gel will stay good over time. Evaluations at 0, 1, 3, and 6 months included organoleptic characteristics, physicochemical parameters (pH, moisture content), microbiological analysis, and heavy metal screening.**

Results: **The results of the test are pretty good. Both types of gel, N-Gel and H-Gel were very stable. Did not change much. The color and smell of N-Gel and H-Gel stayed the same. They felt the same too.**

The pH levels of N-Gel and H-Gel were a little different, but not by much. N-Gel had a pH of 4.60 to 4.92. H-Gel had a pH of 4.75 to 5.00.

The amount of moisture in N-Gel and H-Gel went up a bit over time. N-Gel had 79.80 percent moisture at first. It went up to 85.25 percent. H-Gel had 94.09 percent moisture at first. It went up to 97.32 percent. These levels are still okay.

The N-Gel and H-Gel were also checked for bacteria and other bad things. The total count of bacteria in N-Gel and H-Gel was very low which is what we want. There were no fungi or other harmful organisms found in N-Gel and H-Gel. This is according to the rules of the Ayurvedic Pharmacopoeia, which says that there should be, then 10^3 CFU/g of bacteria. However, heavy metal analysis revealed mercury contamination in both formulations (N-Gel: 28.10 ppm; H-Gel: 21.35 ppm), attributed to laboratory-scale preparation.

Conclusion: **The formulations were pretty stable when we tested them under conditions. We did not see changes in terms of microbiology, chemistry or physical properties. The formulations should last for least two years if we store them properly. Before we start making them on a large scale, we need to make sure they do not have any heavy metals, in them. We have to improve our manufacturing process to get rid of the metals and then do more long-term tests to confirm that the formulations are stable.**

***Keywords*— Accelerated stability testing, Ayurvedic formulations, herbal gel stability, shelf-life determination, *Panchavalkala*.**

I. INTRODUCTION

The ability of a drug product to maintain its identity, potency, and purity within predetermined bounds over the course of its shelf life is known as pharmaceutical stability, which is a crucial quality attribute [1]. Each and every consumable product eventually deteriorates including pharmaceutical products. But a vast difference is observed in the rate of deterioration. Contemporary or modern dosage forms can remain stable and potent for several years. While, Ayurvedic preparations like *Swarasa*, *Kwatha*, etc. need to be consumed as soon as they get formulated or withing 24 hours [2].

Laws governing Ayurvedic, Siddha, and Unani (ASU) medications have changed considerably in India. In order to determine shelf-life period, stability evaluation is required under Rule 16B of the Drugs and Cosmetics Act, which was amended in 2005, 2008, and 2009 to impose mandatory shelf-life requirements [6, 7]. Even the World Health Organization (WHO) offers in-depth guidelines for stability testing for herbal medicines. These can be considered as reference standard worldwide [5].

Stability study includes different protocols like accelerated testing (40°C/75% RH), long-term or real-time testing, intermediate testing and stress testing [8,9]. Although they do not specifically address contemporary stability concepts, classical Ayurvedic texts make reference to drug potency principles (*Saveeryata Avadhi*) and storage conditions (*Agni-Salil-Sweda-Dhuma* protection) in order to preserve therapeutic efficacy [2, 10].

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Modern pharmaceutical science focuses on single active principle. While Ayurvedic, Siddha and Unani formulations are usually having polyherbal composition and possessing synergistic effects. Thus, it becomes challenging to convert them into modern dosage form [12].

There are particular difficulties in modernizing Ayurvedic formulations. Because ASU medicines are polyherbal and have synergistic effects, traditional pharmaceutical approaches that concentrate on single active markers are frequently inappropriate [12]. Accordingly, a holistic evaluation encompassing physicochemical, microbiological and phytochemical parameters is essential to ensure a comprehensive characterization of the formulation.

Panchavalkala is an established Ayurvedic combination which is supposed to be used in the form of decoction and possessing antimicrobial and *Vranaropana* (wound healing) properties. It is made up of the bark of five Ficus species [13,14].

In the Carbopol gel base, *Panchavalkala* was incorporated in combination with either *Nimba* (*Azadirachta indica*), *Haridra* (*Curcuma longa*) or *Kumari* (*Aloe vera*) to formulate the respective polyherbal gels. Compared to daily-prepared liquids, converting these conventional decoctions into gel dosage forms offers benefits like ease of application, dose uniformity, and improved patient compliance [18]. Present study was aimed to perform an accelerated stability evaluation of above stated innovative Ayurvedic wound healing gels to comply regulatory specifications and commercial viability.

II. Materials & Methods

Formulation Composition

Two different gels were formulated using classical Ayurvedic methods for *Kwatha* (decoction) as per *Sharangadhara Samhita* [19] combined with gel technology of modern pharmaceutics [18].

- N-Gel Composition: *Panchavalkala Kwatha* + *Nimba Kwatha* + *Kumari Swarasa* (98%); Carbopol 940 (2%); Triethanolamine (0.2%); Methyl paraben + Propyl paraben (0.2%).
- H-Gel Composition: *Panchavalkala Kwatha* + *Haridra Kwatha* + *Kumari Swarasa* (98%); Carbopol 940 (2%); Triethanolamine (0.2%); Methyl paraben + Propyl paraben (0.2%).

The pH was adjusted to physiologically acceptable levels (6–7) during preparation.

Study Design and Storage Conditions

Accelerated stability studies were conducted in accordance with WHO Technical Report Series No. 953 [5], Schedule-T of the Drugs and Cosmetics Act [6] and ICH guidelines adapted for herbal medicines [20].

- Storage Conditions: 40 ± 2°C and 75 ± 5% RH in a validated stability chamber.
- Duration: Six months.
- Sampling Intervals: Time 0 (Initial), 1 month, 3 months, and 6 months.
- Replicates: Triplicate samples were maintained for each formulation.

Stability Evaluation Parameters

Organoleptic evaluation, visual and sensory assessments were performed for color, odor, appearance (homogeneity, phase separation) and consistency. Changes were documented against the initial sample.

Physicochemical Analysis:

- *pH Determination:* A 1% w/v aqueous solution was prepared and equilibrated at 25 ± 2°C. pH was measured using a calibrated digital pH meter (standardized with pH 4.0, 7.0, and 9.2 buffers) [21].
- *Moisture Content by Karl Fischer Titration:* An accurately weighed sample (1–2 g) was titrated with Karl Fischer reagent in anhydrous methanol. The endpoint was detected via biamperometric indication. Moisture content was calculated as:

  Moisture Content (%) = W V×F×100

  Where,

  V= Volume of reagent (ml),

  F= Factor (mg water/ml), and

  W= Sample weight (mg) [22].

Microbiological Analysis Tests were performed under aseptic conditions following Ayurvedic Pharmacopoeia of India (API) standards [23].

- *Total Aerobic Microbial Count (TAMC):* Using the pour plate method with Soybean Casein Digest Agar (SCDA), incubated at 30–35°C for 48–72 hours. Expressed as CFU/g.
- *Total Yeast and Mold Count (TYMC):* Using Sabouraud Dextrose Agar (SDA), incubated at 20–25°C for 5–7 days. Expressed as CFU/g.
- *Pathogen Testing:* Enrichment and selective plating for Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Salmonella spp.

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Heavy Metal Analysis Screening for Lead (Pb), Cadmium (Cd), Mercury (Hg), and Arsenic (As) was conducted at the initial time point using validated methods (AAS or ICP-MS) [24].

Statistical Analysis:

Data were expressed as mean ± standard deviation of triplicate determinations. Significant changes were defined as variations exceeding ±10% from initial values for quantitative parameters.

Results

Organoleptic Stability: Both N-Gel and H-Gel maintained their original characteristics throughout the six-month period.

- Color: Brown gel (No change).
- Odor: Characteristic herbal odor (No change).
- Appearance: Homogeneous (No phase separation).
- Consistency: Smooth gel (No syneresis).

Physicochemical Stability

Table 1:
pH values during accelerated stability study (40°C, 75% RH)

Formulation	Initial	1 Month	3 Months	6 Months	Total Change	% Change
N-Gel	4.60	4.58	4.85	4.92	+0.32	+6.96%
H-Gel	4.75	4.72	5.03	5.00	+0.25	+5.26%

The slight increase in pH remained well below the 10% significance threshold and within the acceptable range for topical applications.

Table 2:
Moisture content during accelerated stability study

Formulation	Initial (%)	1 Month (%)	3 Months (%)	6 Months (%)	Total increase (%)
N-Gel	79.80	81.48	82.94	85.25	+5.45
H-Gel	94.09	95.20	96.31	97.32	+3.23

Moisture content increased due to the hygroscopic nature of Carbopol and polysaccharides, but no physical instability was observed.

Microbiological Stability

Table 3:
Total aerobic microbial count (CFU/g)

Formulation	Initial	6 Months	Change	Accepted limit (API)	Compliance
N-Gel	36×10^2	56×10^2	+55.6%	$\leq10^3$	Pass
H-Gel	41×10^2	69×10^2	+68.3%	$\leq10^3$	Pass

Total fungal counts were absent throughout the study. Testing for specified pathogens (E. coli, P. aeruginosa, S. aureus, Salmonella spp.) yielded negative results at both initial and final time points.

Heavy Metal Analysis

Table 4:
Heavy metal content in formulations

Metal	N-Gel (ppm)	H-Gel (ppm)	Accepted limit (ppm)	Compliance
Lead (Pb)	Not detected	Not detected	≤10	Pass
Cadmium (Cd)	Not detected	Not detected	≤0.3	Pass
Arsenic (As)	Not detected	Not detected	≤3	Pass
Mercury (Hg)	28.10	21.36	≤1	Fail

Mercury levels significantly exceeded pharmacopeial limits in both formulations.

III. Discussion

Physical and Chemical Stability:

Formulations showed considerable physical stability under accelerated conditions (40°C, 75% RH). 2% Carbopol matrix successfully structured the herbal extracts as there was no phase separation or syneresis observed [18]. A strong intrinsic buffering system is suggested by the small pH variation (<7%), which is probably caused by the herbal components and the equilibrium between carbopol and triethanolamine. The pH range of 4.58–5.03 ensures the efficacy of paraben preservatives and is ideal for skin compatibility (skin pH 4.5–5.5) [25, 26].

Slow rise in moisture content is observed (3-5%). Probable reason for this rise could be hygroscopic nature of carbopol, *Aloe vera* polysaccharides and herbal tannins [27]. In spite of minor rise in moisture content, no physical degradation or microbial growth was observed. However, to prevent any damage due to moisture intrusion under ambient conditions, commercial distribution must be done in moisture-barrier packing like aluminium tubes.

Microbiological Safety:

The microbiological data confirms the adequacy of the preservative system (0.2% methyl and propyl paraben). Total bacterial counts increased modestly but remained well within the API limit of 10^3 CFU/g. The absence of fungal growth is particularly notable given the high humidity stress, suggesting effective antifungal activity and low water activity within the gel matrix.

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The consistent absence of pathogens (*E. coli*, *P. aeruginosa*, *S. aureus*, *Salmonella*) indicates good manufacturing practices during preparation, which is critical for topical wound healing products applied to compromised skin [28].

Critical Quality Concern: Mercury Contamination:

The detection of mercury (N-Gel: 28.10 ppm; H-Gel: 21.35 ppm) represents a significant deviation from WHO guidelines (≤1 ppm) [29]. This contamination is unlikely to originate from the raw botanicals (*Panchavalkala, Nimba, Haridra, Kumari*) as they are not known mercury accumulators.

- *Probable Sources:* The likely sources are laboratory equipment (e.g., mercury thermometers, older analytical instruments) or cross-contamination during small-scale preparation.
- *Toxicological Impact:* Mercury at these levels poses risks of skin sensitization, percutaneous absorption, and systemic toxicity [30].
- *Remedial Actions:* Immediate corrective actions are required, including auditing equipment, replacing mercury-based instruments, implementing GMP-compliant dedicated manufacturing lines, and rigorous raw material screening before commercial production.

Shelf-Life Projection:

Using the Arrhenius equation, the accelerated conditions (40°C) represent an approximately 2.5–3× acceleration factor compared to ambient storage (25°C) [31]. Given the minimal degradation observed over six months under stress, a conservative shelf-life projection of 24 months at 25°C/60% RH is justified. This represents a 730-fold improvement in shelf life compared to traditional *Kwatha* preparations (<1 day) [2], successfully fulfilling the goals of modernization outlined in Rule 161B.

IV. Conclusion

Present study has enough evidences that support stability of novel Ayurvedic wound healing gel. Both gel formulations, N-Gel and H-Gel, demonstrated considerable physical, chemical, and microbiological stability over six months duration under accelerated conditions. The preservative system found to remain effective in stated novel Ayurvedic wound healing gels. The projected shelf-life is a minimum two years duration under recommended storage specifications. But, the mercury contamination is a matter of concern for commercialization. This can be eliminated by proper GMP implementation and manufacturing optimization.

Once resolved, these formulations represent a viable, stable integration of traditional Ayurvedic therapeutics into modern pharmaceutical dosage forms.

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VI. Conflict Of Interest

The authors declare no conflict of interest.

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