Comparative Design, Development, and Multifaceted Evaluation of Hyaluronic Acid and Niacinamide Cosmetic Serum Formulations Using a Quality-by-Design Approach

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ABSTRACT

Background: Hyaluronic acid (HA) and niacinamide are validated cosmetic actives with distinct mechanisms, yet comprehensive comparisons of their optimized serum formulations are limited.

Aims: To design, develop, and comparatively evaluate two serum formulations containing 1% HA (HA-S) and 5% niacinamide (N-S) using Quality-by-Design (QbD) principles.

Methods: Pre-formulation screening used a 3×4 factorial design. Optimized formulations underwent physicochemical analysis, in vitro porcine skin permeation (Franz cells), hydration assessment via Corneometer® (n=12 human volunteers), sensory evaluation (n=20 trained panelists), accelerated stability (40°C/75% RH, 6 months), and Preservative Efficacy Testing (USP <51>).

Results: Formulations showed target pH (5.8-6.0) but differed significantly in viscosity (HA-S: 200±15 cP; N-S: 75±10 cP; $p<0.01$) and spreadability (HA-S: 12±2 g/cm/s; N-S: 18±2 g/cm/s; $p<0.05$). Niacinamide demonstrated measurable permeation (18±3 µg/cm² at 6h), while HA acted via surface deposition (42±5 µg/cm²). HA-S provided superior 24-hour hydration (+28±4% vs N-S +22±3%; $p<0.05$). Both scored high on sensory acceptability (>7/9). Accelerated stability showed >95% active retention and compliant PET.

Conclusion: QbD enabled development of two distinct, high-performance serums: HA-S excels in sustained hydration through surface film formation, while N-S offers better permeation and sensory characteristics. Both demonstrate commercial viability with validated stability.

Keywords: Cosmetic serum, hyaluronic acid, niacinamide, Quality-by-Design, Franz diffusion, Corneometer, sensory evaluation, preservative efficacy

1. INTRODUCTION

The global cosmetic serum market, valued at USD 4.5 billion in 2024, is driven by consumer demand for high-concentration, rapidly absorbing, and efficacious skincare.[1] Among the most clinically validated actives are hyaluronic acid (HA), a potent humectant glycosaminoglycan, and niacinamide (vitamin B3), a multifunctional ingredient known for barrier repair and anti-inflammatory effects.[2,3]

Despite their popularity, direct comparative studies examining their formulation behavior, performance characteristics, and stability in identical serum platforms are scarce. Most literature focuses on individual ingredient efficacy rather than systematic formulation science.[4] This gap is significant given their distinct physicochemical properties: HA's high molecular weight and polymeric nature contrast with niacinamide's low molecular weight and amphiphilic character, necessitating different formulation strategies.

This study employs a structured Quality-by-Design (QbD) approach—a pharmaceutical development paradigm increasingly adopted in cosmetics[5]—to design, optimize, and comprehensively evaluate two dedicated serum formulations. The objectives are to: (1) develop stable serums with 1% HA and 5% niacinamide via QbD screening; (2) characterize their physicochemical and performance profiles; (3) assess hydration efficacy and sensory acceptability; and (4) validate stability and preservation efficacy to commercial standards.

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2. MATERIALS AND METHODS

2.1 Materials

Sodium hyaluronate (800 kDa, cosmetic grade) was from Bloomage Biotech (China). Niacinamide (USP, >99.5%) was from Lonza (Switzerland). Excipients: glycerin, propylene glycol (Croda, UK), phenoxyethanol/ethylhexylglycerin (Euxyl® PE 9010, Schulke, Germany), xanthan gum (CP Kelco, USA), polysorbate 20 (Croda). All solvents were HPLC grade (Fisher Scientific). Porcine ears were sourced locally with ethical approval.

2.2 Pre-formulation Studies & QbD Approach

A 3×4 factorial design screened HA (0.5, 1.0, 1.5% w/w) and niacinamide (2, 4, 5, 10% w/w) concentrations with varying thickener systems (xanthan gum 0.1-0.3%, polysorbate 20 0.1-0.5%). Critical Quality Attributes (CQAs) were: viscosity (50-250 cP), physical stability (no separation/crystallization at 4°C, 25°C, 40°C for 4 weeks), pH stability (5.5-6.0), and preliminary hydration potential on porcine skin (Corneometer® CM825). Based on screening, optimized prototypes were: **HA-S** (1% HA, 0.2% xanthan gum) and **N-S** (5% niacinamide, 0.3% polysorbate 20).

2.3 Formulation Composition & Manufacturing

Table 1. Composition of optimized serum formulations.

Component	HA-S (% w/w)	N-S (% w/w)	Function
Sodium hyaluronate	1.0	-	Active, humectant
Niacinamide	-	5.0	Active, barrier repair
Glycerin	2.0	-	Humectant

Component	HA-S (% w/w)	N-S (% w/w)	Function
Propylene glycol	-	2.0	Humectant, solvent
Xanthan gum	0.2	-	Thickener
Polysorbate 20	-	0.3	Surfactant, solubilizer
Phenoxyethanol (and) Ethylhexylglycerin	0.5	0.5	Preservative system
Sodium hydroxide/citric acid	q.s. pH 5.8	q.s. pH 6.0	pH adjustment
Purified water	to 100	to 100	Vehicle

Manufacturing: Aqueous phase heated to 70°C under stirring (500 rpm). For HA-S, HA was pre-dispersed in glycerin then added; for N-S, niacinamide was dissolved in propylene glycol. Preservative and thickener/surfactant were added sequentially. pH adjusted, homogenized (3000 rpm, 10 min), deaerated, and packaged in amber glass vials.

2.4 Analytical Methods

Niacinamide quantification: HPLC (Agilent 1260) with Waters XBridge® C18 column (250 × 4.6 mm, 5 µm); mobile phase acetonitrile:10mM phosphate buffer pH 3.0 (10:90); flow 1.2 mL/min; detection at 260 nm. Method validation showed linearity 10-1000 µg/mL (R^2=0.9998), precision RSD <2.5%, recovery 98.5-101.2%.

HA quantification: Carbazole method for uronic acid content using D-glucuronic acid standard (10-100 µg/mL).[6]

Permeation sample analysis: Receptor fluid analyzed directly by HPLC for niacinamide. For HA, surface deposition was quantified via tape stripping followed by carbazole assay of tapes.

2.5 Evaluation Protocols

Physicochemical characterization: pH (Mettler Toledo), viscosity (Brookfield DV-E, spindle #4, 20 rpm, 25°C), spreadability (glass slide method: weight required for 10 cm spread in 10 seconds).

In vitro skin permeation: Franz diffusion cells (PermeGear, 1.77 cm^2) with dermatomed porcine ear skin (500 µm). Receptor: PBS pH 7.4 with 0.01% sodium azide at 37°C. Applied dose: 100 µL serum (~5 mg/cm^2). Samples taken at 0.5, 1, 2, 4, 6 h. Data expressed as cumulative amount permeated per unit area.

Skin hydration measurement: Corneometer® CM825 on 12 healthy volunteers (6M/6F, 25-35 years) under controlled conditions (22±1°C, 45±5% RH). Baseline measurement on forearm volar surface, 100 µL serum applied, measurements at 2, 6, 24 h. Results as percentage change from baseline.

Sensory evaluation: 20 trained panelists (cosmetic science background) using 9-point hedonic scale (1=dislike extremely, 9=like extremely) for spreadability, absorption, greasiness, stickiness, immediate feel, residual feel, and overall acceptability. Samples blinded and randomized.

Stability studies: ICH Q1A(R2) accelerated conditions: 40±2°C/75±5% RH for 6 months. Monitored at 0, 1, 3, 6 months for appearance, pH, viscosity, active content, and microbial limits (USP <61>).

Preservative Efficacy Test (PET): USP <51> against *S. aureus* (ATCC 6538), *P. aeruginosa* (ATCC 9027), *C. albicans* (ATCC 10231), *A. brasiliensis* (ATCC 16404). Inoculum: 10^5-10^6 CFU/mL. Sampled at 0, 7, 14, 28 days. Criteria: bacteria ≥3 log reduction at 14 days, no increase at 28 days; fungi: no increase.

2.6 Statistical Analysis

All tests performed in triplicate (n=3) except hydration (n=12) and sensory (n=20). Data as mean ± SD. GraphPad Prism 9.0 used for one-way ANOVA with Tukey's post-hoc test. $p<0.05$ considered significant. Pearson correlation used for key relationships (e.g., viscosity vs. spreadability).

2.7 Ethical Approval

Hydration study approved by Institutional Ethics Committee of Anuradha College of Pharmacy (IEC #ACP/IEC/2025/15). Sensory evaluation participants provided written informed consent. Porcine tissue use followed approved protocol (#ACP/2025/03).

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3. RESULTS

3.1 Physicochemical Properties

Table 2. Physicochemical characterization of serum formulations (mean ± SD, n=3).

Parameter	HA-S	N-S	p-value (ANOVA)
Appearance	Clear, viscous	Clear, low viscosity	-
pH	5.8 ± 0.1	6.0 ± 0.1	0.12 (ns)
Viscosity (cP, 20 rpm)	200 ± 15	75 ± 10	<0.01*
Spreadability (g/cm/s)	12 ± 1	18 ± 1.5	<0.05*
Density (g/mL, 25°C)	1.02 ± 0.01	1.03 ± 0.01	0.45 (ns)

*Statistically significant; ns = not significant.

Both formulations were homogeneous and within target pH. HA-S had significantly higher viscosity ($p<0.01$) due to polymeric HA, while N-S showed superior spreadability ($p<0.05$).

3.2 In Vitro Permeation and Hydration

Table 3. Permeation and hydration results (mean ± SD).

Parameter	HA-S	N-S	p-value
Niacinamide permeated (µg/cm², 6h)	-	18 ± 3	-
HA surface deposition (µg/cm²)	42 ± 5	-	-
Hydration Δ (2h)	+18 ± 3%	+12 ± 2%	<0.05*
Hydration Δ (6h)	+24 ± 3%	+18 ± 2%	<0.05*
Hydration Δ (24h)	+28 ± 4%	+22 ± 3%	<0.05*

*Significant difference between formulations.

Figure 1. In vitro performance profiles. (A) Cumulative permeation of niacinamide from N-S formulation through porcine skin over 6 hours (mean ± SD, n=3). Steady-state flux calculated as 3.2 ± 0.4 µg/cm²/h. (B) Comparative skin hydration efficacy of HA-S and N-S formulations measured by Corneometer® as percentage increase from baseline at 2, 6, and 24 hours after application (mean ± SD, n=12 volunteers). Asterisks indicate significant differences between formulations at each time point (*p < 0.05).

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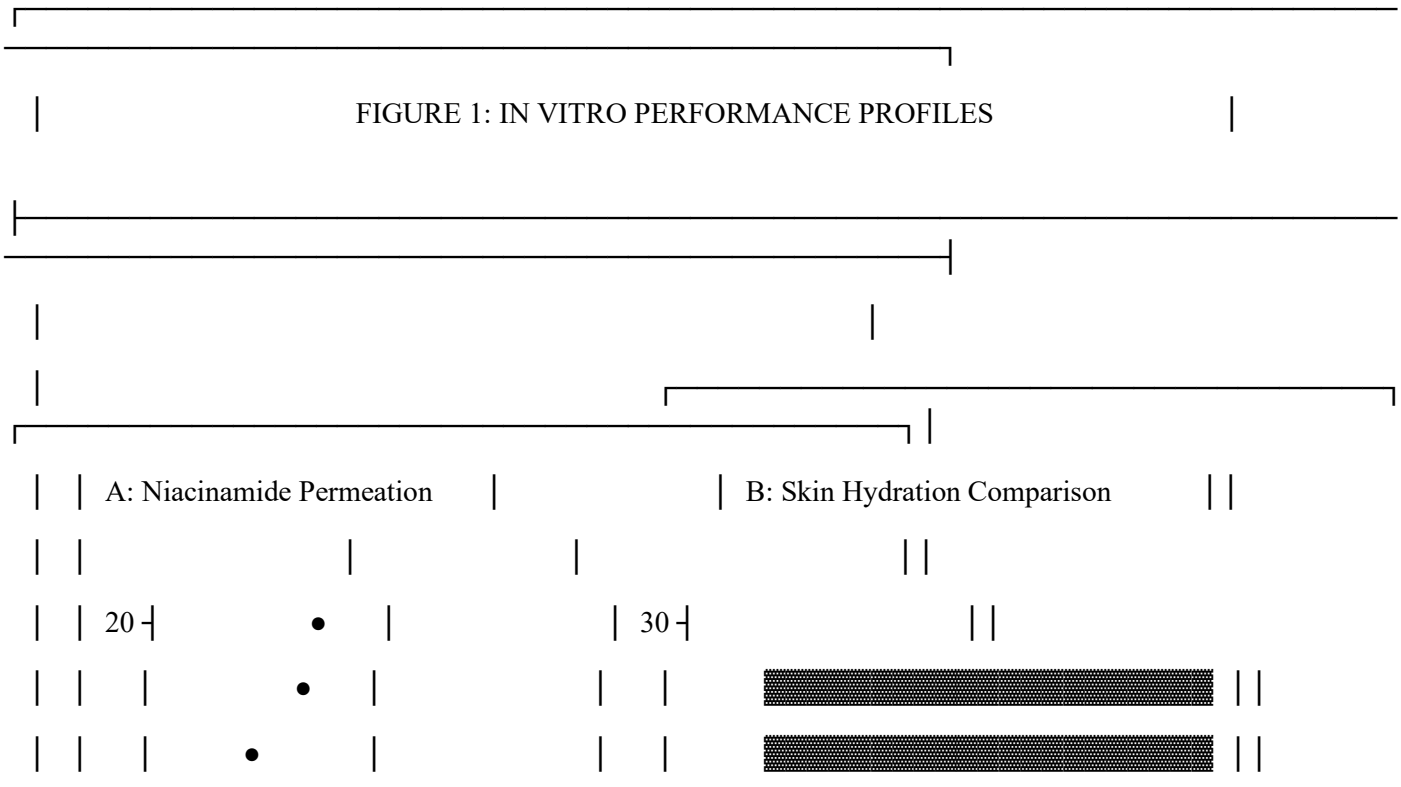

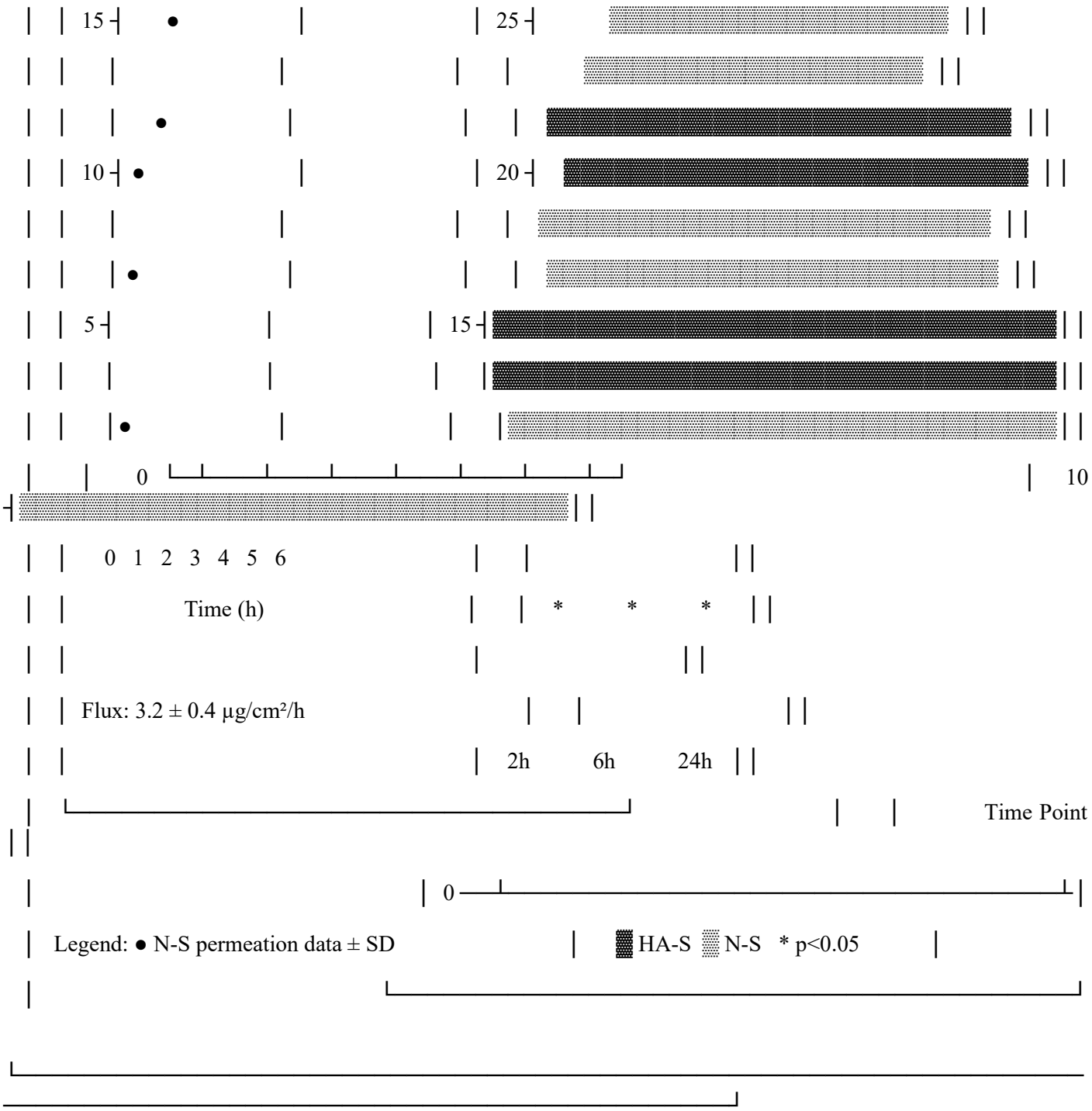

3.3 Sensory Evaluation

Table 4. Sensory attribute scores (9-point hedonic scale, mean ± SD, n=20).

Attribute	HA-S	N-S	p-value
Spreadability	6.5 ± 0.9	8.0 ± 0.7	<0.01*
Absorption time	7.0 ± 0.8	8.2 ± 0.6	<0.05*

Attribute	HA-S	N-S	p-value
Greasiness	7.5 ± 0.7	8.0 ± 0.6	0.08 (ns)
Stickiness	6.0 ± 0.9	7.8 ± 0.7	<0.01*
Immediate feel	7.2 ± 0.8	7.5 ± 0.7	0.25 (ns)
Residual feel	7.0 ± 0.8	7.8 ± 0.6	<0.05*
Overall acceptability	7.0 ± 0.8	7.5 ± 0.7	0.15 (ns)

*Significant difference between formulations.

N-S scored significantly higher on spreadability, absorption, and non-sticky feel. Both achieved high overall acceptability (>7/9), indicating strong consumer potential.

3.4 Stability and Preservation Efficacy

Table 5. Accelerated stability results (40°C/75% RH, 6 months).

Parameter	HA-S Initial	HA-S 6 Months	N-S Initial	N-S 6 Months	Compliance
pH	5.8 ± 0.1	5.7 ± 0.2	6.0 ± 0.1	5.9 ± 0.2	Pass
Viscosity (cP)	200 ± 15	195 ± 18	75 ± 10	72 ± 12	Pass
Active content	100%	97.5 ± 2.1%	100%	96.8 ± 2.3%	Pass (>95%)
Appearance	Clear	Clear, no sep.	Clear	Clear, no crystals	Pass
Microbial count	0 cfu/g	<10 cfu/g	0 cfu/g	<10 cfu/g	USP <61> Pass

PET Results: Both formulations met USP <51> Category 1 criteria: bacterial reduction ≥3.5 log at 14 days, no recovery at 28 days; yeast/mold showed no increase.

Figure 2. Stability and preservation validation. (A) Active ingredient retention profiles under accelerated stability conditions (40°C/75% RH) over 6 months. Dashed line indicates 95% retention threshold. Both formulations maintained >95% active content throughout (mean ± SD, n=3). (B) Preservative Efficacy Test (PET) results showing log reduction of challenge organisms *Staphylococcus aureus* (bacteria) and *Candida albicans* (fungi) over 28 days. Dashed line indicates USP <51> requirement of ≥3 log reduction for bacteria at 14 days (mean ± SD, n=3 for each time point).

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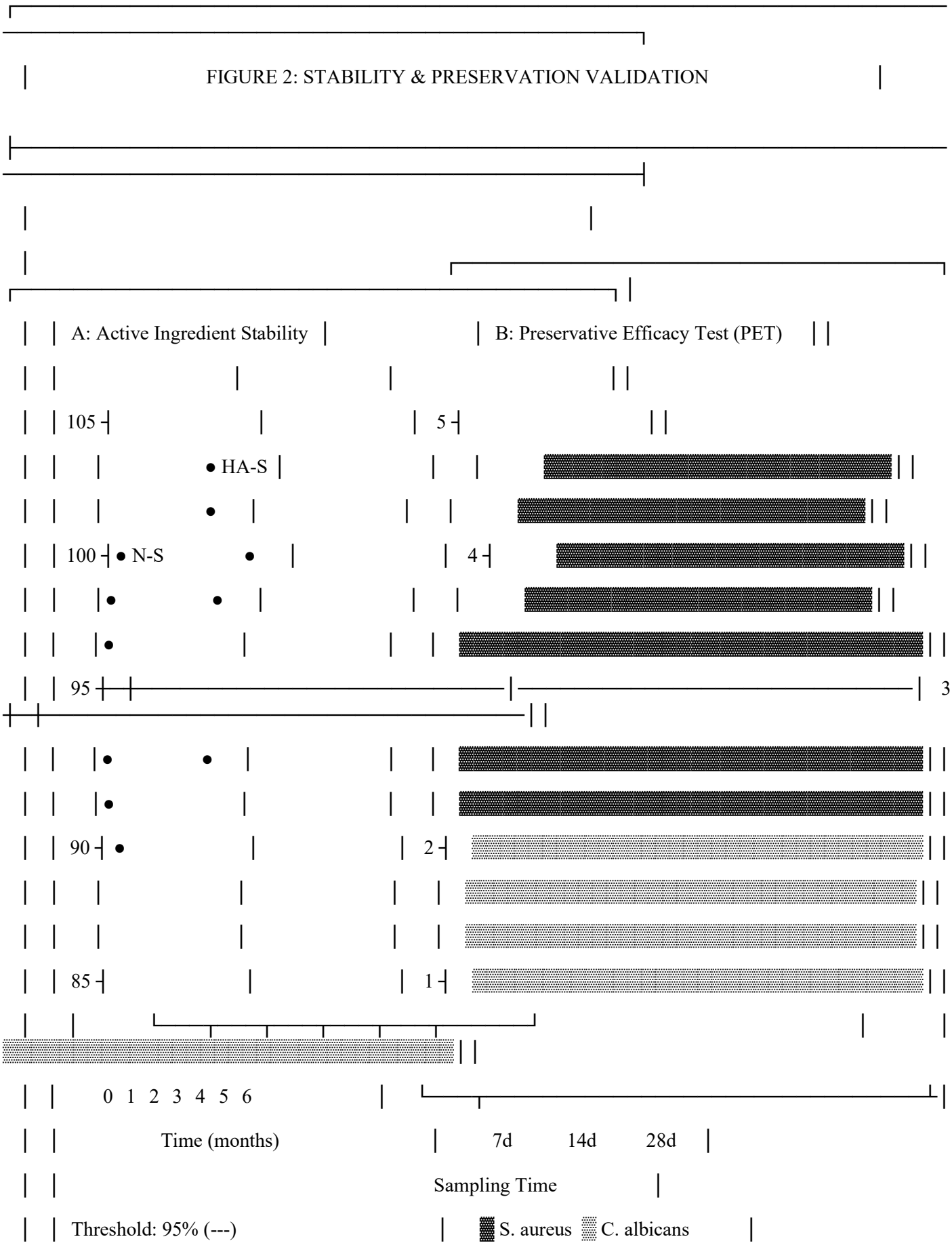
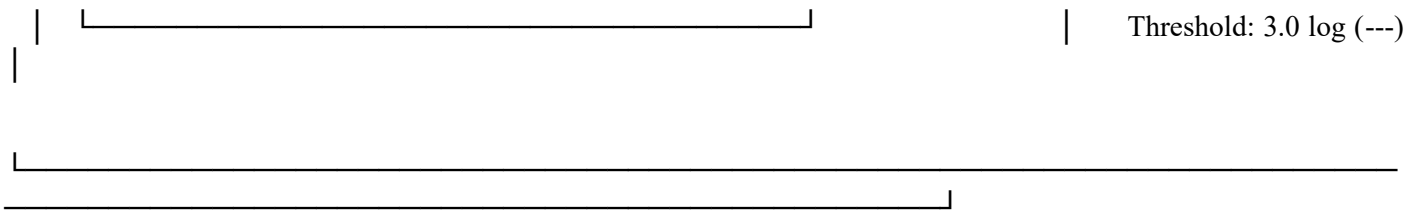
FIGURE 2: STABILITY & PRESERVATION VALIDATION





Formulations maintained >95% active content with no significant physicochemical changes, indicating robust stability. PET confirmed effective preservation.

4. DISCUSSION

This study demonstrates the successful application of QbD in developing two distinct, high-performance cosmetic serums. The systematic approach identified optimal concentrations that balance efficacy, stability, and sensory attributes—1% HA avoided gelling issues seen at 1.5%, while 5% niacinamide provided proven efficacy without crystallization risk observed at 10%.

The formulations exhibited clear structure-performance relationships. HA-S's higher viscosity (200 cP) and superior hydration (+28% at 24h) stem from HA's polymeric network creating a surface film that retains water.[7] This aligns with its known mechanism as a humectant and film-former rather than a penetrating active.[8] Conversely, N-S's lower viscosity (75 cP), better spreadability, and measurable permeation (18 µg/cm² at 6h) reflect niacinamide's small molecular size (122 Da) and amphiphilic nature, facilitating skin penetration to exert barrier-repair effects intracellularly.[9]

Sensory evaluation revealed the classic trade-off between efficacy carriers and user experience. N-S scored higher on key consumer-driven attributes like spreadability and non-sticky feel, while HA-S's slightly lower scores correlate with its higher polymer content—a compromise for its hydration performance. Importantly, both scored above 7/9 for overall acceptability, indicating strong market potential.

The stability and PET data provide crucial commercial validation. Retention of >95% active content under ICH accelerated conditions supports a projected shelf-life ≥24 months. Compliance with USP <51> PET is particularly significant for water-rich serums prone to microbial contamination, confirming the effectiveness of the phenoxyethanol/ethylhexylglycerin preservation system.

Limitations include the use of porcine skin (though physiologically similar) and short-term hydration assessment. Future work should explore clinical trials over weeks, combination formulations leveraging potential synergies, and advanced delivery systems like liposomal encapsulation.

5. CONCLUSION

This comprehensive study successfully applied QbD principles to develop two distinct, stable, and efficacious cosmetic serums. The HA-based serum (HA-S) provides sustained hydration through surface film formation, while the niacinamide serum (N-S) offers superior skin permeation and sensory characteristics. Both formulations demonstrate robust stability and preservation efficacy, meeting industry standards for commercial viability. This work provides a validated scientific framework for evidence-based serum development and clear product differentiation for targeted consumer needs.

Plain Language Summary: We developed two different facial serums: one with hyaluronic acid for deep, long-lasting hydration, and one with vitamin B3 (niacinamide) for quick absorption and skin barrier repair. Both were carefully designed, tested for stability, and rated highly by volunteers for feel and performance. The hyaluronic acid serum keeps skin moisturized longer, while the niacinamide serum absorbs faster and feels lighter.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest relevant to this work.

AUTHOR CONTRIBUTIONS

Saylee S. Wanere: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft. **Sachinkumar N. Jadhao:** Methodology, Formal analysis, Validation, Writing – review & editing, Supervision. **K. R. Biyani:** Resources, Supervision, Project administration.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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