ORIGINAL ARTICLE

Development and Optimization of Polyglutamic acid Enriched Lip Balm Using Experimental Design Approach

Manish D. Baviskar[1], Ruchira M. Gajbhiye[2], Sandeep S. Sonawane[3], Amruta J. Panjabi[2], Nilima A. Thombre[4]

[1]Tri-Pac, Inc., South Bend, Indiana, USA, [2]Department of Cosmetic Technology, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Maharashtra, India, [3]Department of Pharmaceutical Analysis, MET's Institute of Pharmacy, Bhujbal Knowledge City, Nashik, Maharashtra, India, [4]Department of Pharmaceutics, MET's Institute of Pharmacy, Bhujbal Knowledge City, Nashik, Maharashtra, India

Abstract

Introduction: This study aimed to develop a novel topical lip formulation with enhanced hydration, moisturization, and barrier protection, addressing the high susceptibility of lips to dehydration and environmental damage. **Materials and Methods:** The formulation incorporated shea butter as the primary structural excipient, almond oil as an emollient to improve spreadability, peppermint oil for sensory appeal, and polyglutamic acid (PGA) as the principal hydrating agent due to its strong water-binding and film-forming properties. A 2^3 full factorial design was employed, with concentrations of shea butter, almond oil, and PGA as independent variables. pH and spreadability were selected as critical quality attributes. Statistical analysis was performed to assess individual and interaction effects, followed by optimization using a desirability function targeting a pH of 5.5–6.5 and spreadability of 6–9 cm. **Results and Discussion:** PGA significantly influenced formulation pH, while shea butter and almond oil predominantly governed spreadability. The factorial design effectively elucidated the relationships between formulation variables and product performance. Numerical optimization produced an optimized formulation with an overall desirability value of 1.000, indicating optimal physicochemical and application characteristics. **Conclusion:** The optimized PGA–enriched lip formulation demonstrated suitable pH, desirable spreadability, and balanced performance, highlighting the effectiveness of factorial design and desirability-based optimization in advanced lip care product development.

Key words: Desirability function, factorial design, lip balm, optimization, polyglutamic acid

INTRODUCTION

Cosmetic products are extensively utilized for both esthetic enhancement and the preservation of skin health, offering protection against environmental aggressors. Effective lip care is particularly crucial due to the absence of sebaceous glands in the labial mucosa, rendering the susceptibility of the lips to desiccation, chapping, and irritation induced by environmental stressors.[1] Conventional lip balms, which are primarily based on petroleum derivatives, waxes, or synthetic excipients, provide only short-term relief. However, such formulations are often limited in their ability to deliver long-lasting hydration and may even lead to irritation or allergic reactions in individuals with sensitive skin.[2]

Polyglutamic acid (PGA) has recently emerged as a significant biopolymer, demonstrating considerable potential for advanced cosmetic applications. This naturally occurring, water-soluble polypeptide is derived from the microbial fermentation of *Bacillus subtilis* and is recognized for its outstanding hydrating and film-forming capabilities.[3] Structurally, PGA is composed of recurring glutamic acid units interconnected by amide bonds, a configuration that confers a robust capacity for water retention. Research indicates that PGA possesses the ability to sequester several times more water than hyaluronic acid, thereby establishing its position as one of the most efficacious humectants currently recognized.[4]

Address for Correspondence:
Ruchira M. Gajbhiye, Department of Cosmetic Technology, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Maharashtra, India.
E-mail: ruchira.gajbhiye@rcpatelpharmacy.co.in

Received: 13-02-2026
Revised: 18-03-2026
Accepted: 27-03-2026

PGA not only functions as a humectant but also offers additional advantages pertinent to lip care products. It forms a fine, permeable film on the skin surface, which aids in reducing transepidermal water loss and strengthening the skin's intrinsic barrier function.[5] Furthermore, PGA promotes the synthesis of natural moisturizing factors within the stratum corneum, thereby enhancing elasticity and contributing to improved lip softness and comfort.[6,7] Its biodegradability, biocompatibility, and non-irritant nature further support its suitability for cosmetic applications.[8,9]

In light of these attributes, the incorporation of PGA into lip balm matrices represents an innovative strategy for achieving prolonged moisturization, enhanced barrier protection, and improved user compliance.[9,10] However, the performance of such multifunctional formulations is highly dependent on the precise composition and interaction of excipients, necessitating a systematic and statistically driven optimization approach.

In the context of this, the present study employed a 2^3 full factorial experimental design to systematically optimize the lip balm formulation. Shea butter, almond oil, and PGA were selected as independent formulation variables based on their functional roles in providing structural integrity, emolliency, and hydration, respectively. pH and spreadability were identified as critical quality attributes (CQAs) due to their direct relevance to product safety, stability, and consumer acceptability. The factorial design enabled the evaluation of both individual and interactive effects of formulation variables on these responses, thereby facilitating the identification of an optimized formulation with balanced dermatological compatibility and application performance. Accordingly, the current investigation is dedicated to the systematic formulation and comprehensive evaluation of a PGA-enriched lip balm, with the primary objective of substantiating its therapeutic potential as a multifunctional cosmeceutical agent through a statistically validated design and optimization strategy.[11,12]

MATERIALS AND METHODS

Materials

PGA used in the study was obtained via microbial fermentation using *B. subtilis* and was of cosmetic grade and laboratory quality. All other ingredients, shea butter, almond oil, peppermint oil, and polysorbate 80, were procured from the departmental laboratory stock and adhered to analytical or cosmetic grade specifications, making them suitable for formulation development.

Methods

The lip balm was designed as a water-in-oil emulsion system, specifically engineered to enable the stable incorporation of a hydrophilic active ingredient within a predominantly lipophilic matrix. Such a system was selected to ensure prolonged retention on the lip surface, enhanced barrier protection, and sustained moisturization. The oil phase consisted of shea butter, almond oil, oil-soluble pigment, and peppermint oil. Shea butter was employed as the primary structural excipient due to its semi-solid consistency, occlusive nature, and barrier-reinforcing properties contributing to firmness and moisture retention. Almond oil was incorporated as an emollient to enhance spreadability and sensory attributes. Peppermint oil, being lipophilic, was incorporated into the oil phase to impart a cooling and refreshing sensation, thereby improving consumer acceptability. Oil-soluble pigments were included to provide uniform coloration and esthetic appeal.

The aqueous phase comprised PGA, distilled water, and Polysorbate 80. PGA was selected as the principal hydrating and film-forming agent due to its superior water-binding capacity and moisture-retention properties. Polysorbate 80 was included in the aqueous phase to facilitate solubilization and uniform dispersion of PGA within the formulation and to assist in maintaining emulsion stability.

A 2^3 full factorial design was employed to systematically investigate the effect of formulation variables on product performance and to optimize the lip balm composition. Shea butter, almond oil, and PGA were selected as independent variables and evaluated at low level (−1) and at high level (+1) to evaluate its impact on hydration and formulation pH. The selected independent variables and their concentrations are presented in Table 1. All other formulation components were maintained at constant concentrations across all experimental runs. This experimental design resulted in eight formulations (F1-F8), allowing the evaluation of both main effects and interaction effects of the selected variables.

The composition of the lip balm formulation is presented in Table 2.

Preparation of lip balm formulations

The lip balm formulations were prepared using the fusion method. Shea butter and almond oil were accurately

Table 1: Selected independent variables with justification

Independent variable	Low level (%)	High level (%)	Justification
Shea butter (A)	50	75	Provides structural integrity and rigidity
Almond oil (B)	4	5	Modulates emolliency and spreadability
PGA (C)	1.5	2.5	Primary hydrating agent and pH modifier

PGA: Polyglutamic acid

Table 2: Composition of factorial lip balm formulations (F1–F8)

S. No.	Ingredient	Concentration (% *w/w*)
1.	Shea butter*	50–75
2.	Almond oil*	4–5
3.	Polyglutamic acid*	1.5–2.5
4.	Peppermint oil	0.5
5.	Oil-soluble pigments	1.0
6.	Polysorbate 80	1.0
7.	Distilled water	8.0
8.	Beeswax	q.s. to 100

*As per experimental design matrix

weighed, combined, and heated on a water bath at 70–75°C until completely melted. Oil-soluble pigments were then incorporated into the molten oil phase with continuous stirring to obtain a homogeneous oil phase.

The aqueous phase was prepared separately by dissolving PGA and Polysorbate 80 in distilled water and heating the solution to the same temperature as the oil phase. The heated aqueous phase was slowly added to the oil phase under continuous stirring to ensure uniform dispersion of the hydrophilic components within the oleaginous matrix. Peppermint oil was added during the homogenization step to preserve its volatile component.

The resulting matrix was allowed to cool gradually at room temperature with gentle stirring until solidification occurred. The solidified lip balm was transferred into suitable containers and stored at room temperature for further evaluation.

pH and spreadability were identified as CQAs due to their direct influence on product safety, stability, and consumer acceptance. pH was selected to ensure dermatological compatibility with the lip mucosa and to minimize irritation, with an acceptable range aligned to physiological skin pH. Spreadability was chosen as a key performance attribute reflecting ease of application, uniformity of film formation, and sensory perception during use. Based on the evaluation of CQAs, the formulation that met the predefined acceptance criteria for pH and spreadability was identified as the optimized formulation and selected for further characterization and stability assessment.

The obtained optimized formulation was subsequently evaluated for accelerated stability studies over a period of 4 weeks at (4.0 ± 2.0°C, 25.0 ± 3.0°C, and 40.0 ± 2.0°C) to assess the integrity and performance of the formulations under different environmental stresses.

RESULTS AND DISCUSSION

A total of eight lip balm formulations (F1-F8) were prepared using a 2^3 full factorial design with varying concentrations of shea butter, almond oil, and PGA while keeping other excipients constant. No immediate signs of phase separation, grittiness, or visual instability were observed, indicating the suitability of the selected formulation strategy and preparation method. pH and spreadability were identified as CQAs, and the experimental matrix is depicted in Table 3.

Effect of formulation variables on pH and spreadability

The effect of shea butter (A), almond oil (B), and PGA (C) on the CQAs, namely, pH and spreadability was systematically evaluated using 2^3 full factorial design. Statistical tools, including Pareto analysis, analysis of variance (ANOVA), polynomial regression equations, and perturbation plots, were employed to elucidate the significance, direction, and sensitivity of each variable on the responses.

Pareto Analysis revealed a distinct difference in the factors governing pH and spreadability of the lip balm formulation, depicted in Figure 1.

For the pH response, PGA (C) was identified as the only statistically significant factor, as it crossed both the t-value and Bonferroni limits, whereas shea butter (A) and almond oil (B) remained below the significance thresholds. This indicates that variations in PGA concentration (C) had a pronounced effect on pH, while the lipid components exerted a negligible influence within the studied ranges. In contrast, the Pareto chart for spreadability demonstrated that shea butter (A) and almond oil (B) were statistically significant, as both factors exceeded the significance limits. PGA (C), however, did not cross the threshold, suggesting a minimal contribution to spreadability. These findings highlight that pH is primarily controlled by the hydrophilic active, whereas spreadability is predominantly governed by the lipid phase composition.

ANOVA results further substantiated the Pareto findings for both responses. Table 4a and b depict the ANOVA for pH and spreadability, respectively.

For pH, the model was statistically significant, with a high F-value and $P < 0.05$. Among the individual factors, only PGA (C) exerted a significant effect, while shea butter (A) and almond oil (B) were statistically insignificant. The goodness-of-fit parameters (R^2, adjusted R^2, and predicted R^2) were in close agreement, indicating that the model adequately explained the variability in pH and possessed strong predictive capability. A high adequate precision value confirmed a favorable signal-to-noise ratio, validating the robustness of the model. Similarly, for spreadability, ANOVA confirmed the statistical significance of the model. Shea butter (A) and almond oil (B) were identified as significant model terms, while PGA (C) was insignificant. The high R^2 values and close agreement between adjusted and predicted

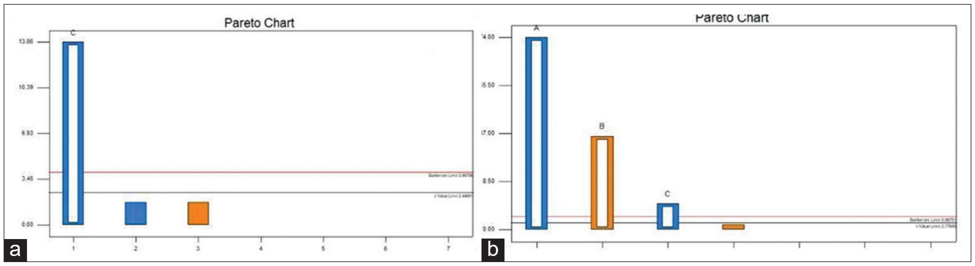

Figure 1: Pareto chart of (a) pH and (b) spreadability

Table 3: Experimental matrix

Formulation No	Shea butter (% w/w)	Almond oil (% w/w)	PGA (% w/w)	pH	Spreadability* (cm)
F1	50	4	1.5	6.4	5.8
F2	75	4	1.5	6.3	3.9
F3	50	5	1.5	6.5	6.7
F4	75	5	1.5	6.4	4.8
F5	50	4	2.5	5.6	5.5
F6	75	4	2.5	5.5	3.7
F7	50	5	2.5	5.7	6.4
F8	75	5	2.5	5.6	4.6

*Glass slide method, 500 g load, 1 min expressed as diameter in cm

Table 4a: ANOVA for pH

Source	Sum of squares	df	Mean square	F-value	*P*-value	
Model	1.28	1	1.28	192.00	<0.0001	Significant
C-PGA	1.28	1	1.28	192.00	<0.0001	
Residual	0.04	6	6.67×10^{-3}			
Corrected total	1.32	7				
Standard deviation			0.082	R^2		0.9697
Mean			6.00	Adjusted R^2		0.9646
CV %			1.36	Predicted R^2		0.9461
Press			0.071	Adeq Precision		19.596

ANOVA: Analysis of variance, CV: Coefficient of variation, df: Degrees of freedom, PGA: Polyglutamic acid

Table 4b: ANOVA for spreadability

Source	Sum of squares	df	Mean square	F-value	*P*-value	
Model	8.59	3	2.86	2290.67	<0.0001	Significant
A - Shea butter	6.85	1	6.85	5476.00	<0.0001	
B - Almond oil	1.62	1	1.62	1296.00	<0.0001	
C - PGA	0.13	1	0.13	100.00	0.0006	
Residual	5×10^{-3}	4	1.25×10^{-3}			
Corrected total	8.60	7				
Standard deviation			0.035	R^2		0.9994
Mean			5.17	Adjusted R^2		0.9990

ANOVA: Analysis of variance, CV: Coefficient of variation, df: Degrees of freedom, PGA: Polyglutamic acid

R^2 indicated good model reliability. Adequate precision values well above the recommended limit further confirmed the suitability of the model for optimization.

The polynomial equation for pH = 6.00–0.45C clearly indicates a negative effect of PGA (C) on pH. An increase in PGA concentration (C) resulted in a measurable decrease

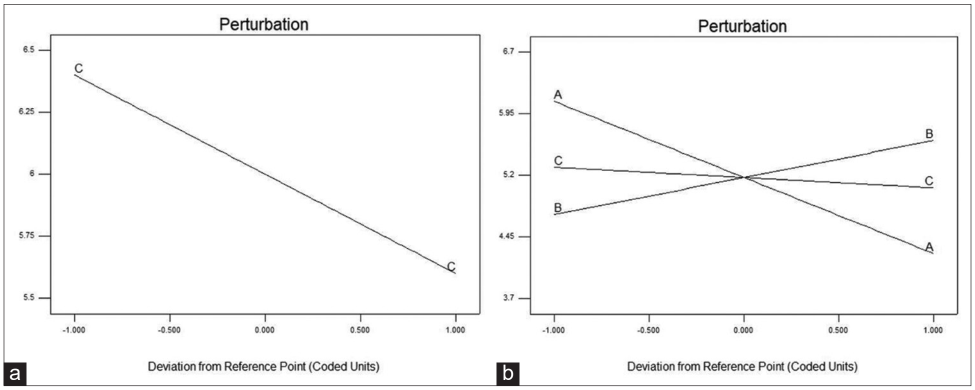

Figure 2: Perturbation plot (a) for pH and (b) for spreadability

in pH. The absence of coefficients for shea butter (A) and almond oil (B) further confirms their negligible influence on pH.

In contrast, the polynomial equation for spreadability = 5.18–0.93A + 0.45B–0.13C, demonstrates that shea butter (A) is the most influential factor with a strong negative effect, followed by almond oil (B) with a positive effect on spreadability. The coefficient for PGA (C) is comparatively small, confirming its minimal role in influencing spreadability. The negative effect of shea butter (A) is associated with increased formulation rigidity at higher concentrations, while the positive contribution of almond oil (B) is due to its low viscosity and emollient nature, which enhances slip and ease of application.

Perturbation plots provided further insight into the sensitivity of the responses to individual formulation variables. For pH, the perturbation curve corresponding to PGA (C) exhibited a steep negative slope, indicating high sensitivity of pH to small changes in PGA concentration (C). For spreadability, the perturbation plot showed a steep negative slope for shea butter (A), signifying a strong inverse relationship with spreadability, and a steep positive slope for almond oil (B), confirming its dominant role in improving spreadability. The perturbation curve for PGA (C) remained relatively flat, indicating negligible sensitivity of spreadability to PGA concentration (C). The perturbation plots for pH and for spreadability are presented in Figure 2a and b, respectively.

Based on the desirability function analysis, wherein pH (5.5–6.5) and spreadability (~5.5–6.5 cm) were assigned equal weight (1) and importance (+++), the formulation that simultaneously satisfied both CQAs with maximum overall desirability (~1.0) was selected as the optimized formulation. Hence, the composition consisting of shea butter 50.5%, almond oil 4.3%, and PGA 2%, provides acceptable pH 6.0 and spreadability 5.9, respectively.

When the optimized formulation underwent stability assessment under refrigerated (4°C), ambient (25°C), and elevated (40°C) temperature conditions, no phase separation was evident, with retention of fragrance, color, and spreadability observed throughout the evaluation period. A minor shade darkening occurred solely at elevated temperatures, while pH values remained within acceptable limits. Table 5 depicts the results of stability studies.

Table 5: Stability studies of optimized formulation

Parameter	4°C	25°C	40°C
Color	Natural	Natural	Slightly darker
Odor	Pleasant	Pleasant	Pleasant
Melting behavior	Solid	Solid	Slightly soft
Spreadability	Uniform	Uniform	Uniform
pH	5.8	6.0	5.9

CONCLUSION

The present study demonstrates the systematic design, development, and optimization of PGA -enriched lip balm using an experimental design approach. A 2^3 full factorial design was employed to investigate the influence of shea butter, almond oil, and PGA on CQA - pH and spreadability. Statistical evaluation was performed using Pareto analysis, ANOVA, polynomial regression equations, and perturbation plots revealed that PGA was the most significant factor affecting pH, while shea butter and almond oil predominantly governed spreadability. The developed statistical models exhibited high goodness-of-fit, predictive capability, and compliance with ANOVA assumptions, confirming their reliability for formulation optimization. Further numerical optimization using the desirability function approach enabled simultaneous optimization of pH and spreadability within predefined acceptable ranges. Overall, the study highlights the effectiveness of integrating factorial design and desirability-based optimization in cosmetic formulation development. The optimized PGA -based lip balm offers enhanced hydration potential, balanced sensory attributes, and improved consumer acceptability, thereby demonstrating promising potential as a multifunctional cosmeceutical lip care product.

REFERENCES

1. Jackson EM. Lip care products. In: Schlossman ML, editor. The Chemistry and Manufacture of Cosmetics. 4th ed. Carol Stream, IL: Allured Publishing; 2009. p. 1153-72.
2. Sankpal RM, Kadam SR, Aswale NS, Navale SS. Natural lip balm. Int J Adv Res Sci Commun Technol 2022;2:450-3.
3. Shih IL, Van YT. The production of poly-(gamma-glutamic acid) from microorganisms and its various applications. Bioresour Technol 2001;79:207-25.
4. Shaikh N, Hingane LD. Formulation and characterization of lip balm from beetroot. Int J Res Trends Innov 2022;7:1224-32.
5. Tao L, Long H, Zhang J, Qi L, Zhang S, Li T, *et al*. Preparation and coating application of γ-polyglutamic acid hydrogel to improve storage life and quality of shiitake mushrooms. Food Control 2021;130:108404.
6. Urai M, Aizawa T, Nakajima M, Sunairi M. An anionic polymer incorporating low amounts of hydrophobic residues is a multifunctional surfactant. Part 2: Emulsification, moisture absorption, and moisture retention of alkyl esterified poly-γ-glutamic acid. Adv Chem Eng Sci 2015;5:181-91.
7. Panjabi AJ, Gajbhiye RM. Polyglutamic acid-infused lip gloss: Enhancing shine with non-sticky hydration. J Sci Res Int 2025;11:222-30.
8. Serra M, Gudiña EJ, Botelho C, Teixeira JA, Barros AN. Polyglutamate: Unleashing the versatility of a biopolymer for cosmetic industry applications. Cosmetics 2024;11:76.
9. Johnson LC, Akinmola AT, Scholz C. Poly(glutamic acid): From natto to drug delivery systems. Biocatal Agric Biotechnol 2022;40:102292.
10. Al-Wahili M, Al-Manhel A, Al-Ali R, Altemimi A. Poly-gamma-glutamic acid: A comprehensive overview of biosynthesis, characteristics, and emerging applications. J Microbiol Biotechnol Food Sci 2023;13(3):e10075.
11. Elbanna K, Alsulami FS, Neyaz LA, Abulreesh HH. Poly (γ) glutamic acid: A unique microbial biopolymer with diverse commercial applicability. Front Microbiol 2024;15:1348411.
12. Yu Z, Wei Y, Fu C, Sablani SS, Huang ZE, Han C, *et al*. Antimicrobial activity of gamma-poly (glutamic acid), a preservative coating for cherries. Colloids Surf B Biointerfaces 2023;225:113272.

Source of Support: Nil. **Conflicts of Interest:** None declared.