RESEARCH ON FORMULATION AND EVALUATION OF *IN-SITU* GEL OF MINOXIDIL

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Abstract

The research focuses on the formulation and evaluation of *in-situ* gel of minoxidil Androgenic alopecia (AGA) significantly affects both physical appearance and psychological well-being, leading to progressive hair thinning due to genetic and hormonal factors. Minoxidil, an FDA-approved treatment, has long been a cornerstone for addressing AGA. Originally developed for hypertension, it was repurposed after its hair growth-promoting effects were discovered. Despite its effectiveness, conventional minoxidil formulations require frequent applications and can cause scalp irritation, limiting their overall eficacy and patient adherence. Main Body Hair growth cycles through anagen (growth), catagen (transition), and telogen (resting) phases. In AGA, the anagen phase shortens while the telogen phase lengthens, resulting in thinner hair. Minoxidil helps by extending the anagen phase and enlarging hair follicles. However, traditional formulations suffer from rapid solvent evaporation, leading to crystallization on the scalp and irritation. *In-situ* gels offer a novel solution, transforming from liquid to gel upon exposure to physiological conditions like temperature or pH changes. This allows for sustained release of minoxidil, maintaining effective drug concentrations on the scalp for longer periods and reducing the frequency of application. This approach enhances patient compliance and minimizes side effects. Conclusion In-situ gels represent a significant advancement in treating AGA by providing controlled, sustained drug release. They overcome the limitations of conventional minoxidil formulations, improving therapeutic outcomes and patient adherence. As research continues, in-situ gels hold the potential to revolutionize hair loss management.

Keywords: Hair loss, androgenic alopecia, minoxidil, *in-situ* gels, drug delivery systems

1. Introduction

Skin is the largest organ in the human body and serves several vital functions. It acts as a protective barrier, shielding the body from physical harm, pathogens, and UV radiation. Additionally, skin regulates body temperature, helps with the sensation of touch, and plays a role in the synthesis of vitamin D.The skin consists of three main layers: Epidermis , Dermis , Subcutaneous tissue (Hypodermis)

1.1 Androgenetic Alopecia (Male and Female Pattern Baldness): This is the most common form of hair loss and is often hereditary. In men, it typically results in a receding hairline and baldness on the top of the head. In women, it usually leads to thinning of the hair on the crown of the scalp.

1.2 Minoxidil: A topical solution or foam applied to the scalp. It is available over-the-counter and is one of the most common treatments for androgenetic alopecia.

1.3 *In-situ* gel :It refers to a type of gel that forms or undergoes a gelation process directly at the site of application or administration. This is often encountered in pharmaceutical and biomedical applications, where the gel transitions from a liquid to a gel state in response to specific physiological conditions or external triggers. The objective is to provide a controlled and sustained release of active substances.

2. Materials and Methods

2.1 Materials

Gellan gum is a food additive derived from the bacterium Sphingomonas elodea. It's a water-soluble polysaccharide widely used as a thickener, stabilizer, and gelling agent in various food and industrial products. Due to its unique ability to form gels in small amounts, it's a popular alternative to other gelling agents like agar or gelatin.

There are two main types of gellan gum:

- Low-acyl gellan gum: Creates firm, brittle gels.
- High-acyl gellan gum: Forms softer, more elastic gels.

Chemical formula: $C_{12}H_{18}O_9$ (polysaccharide)

Uses

It's found in pharmaceuticals, personal care products, and as a gelling agent in microbiological media.

Carbopol 940 P It belongs to a family of synthetic polymers known as carbomers. Carbopol 940 P is specifically designed for pharmaceutical-grade products, where the "P" denotes its use in pharmaceutical formulations.

Key Properties:

- Thickening agent: Carbopol 940 P is highly efficient in thickening aqueous solutions, even at low concentrations.
- Gel formation: It forms clear, stable gels when neutralized with a base like sodium hydroxide or tri-ethanolamine.
- Stabilizer: It can stabilize emulsions, suspensions, and other formulations.
- High viscosity: It provides high viscosity at a low concentration.

2.2 Minoxidil

Minoxidil is a medication commonly used to treat hair loss, and it is available over-the-counter in various formulations, including topical solutions and foams. Here are key points about minoxidil:

2.2.1 Mechanism of Action:

The exact mechanism of how minoxidil promotes hair growth is not fully understood, but it is believed to increase blood flow to the hair follicles, leading to improved follicular function and stimulation of hair growth.

2.2.2 Application:

Minoxidil is typically applied directly to the scalp in the form of a liquid solution or foam.

It should be applied to a dry scalp and left on for a certain period before washing.

2.3 Detrmination of solubility study

The solubility studies of drug in aqueous and non-aqueous phases are the important properties during formulation consideration and also behavior and transport of drugs in the body. Equilibrium solubility was determined at room temperature, for this, systems of each solvent (DW, Methanol, Ethanol & PBS pH 5.5)

2.4 Prepration of in situ gel

Aqueous solutions of varying concentration containing Carbopol 934P and Gellan gum will be prepared. Dispersion containing carbopol 934P of different concentration (0.2-1.4% w/v) will be initially prepared in deionized water containing 0.17% w/v sodium citrate. After that amounts of calcium chloride (0.05% w/v) will be added into the sol. Drug (2%) will then dissolved in ethanol and will added to the solution. The mixture was stirred by using a magnetic stirrer to ensure thorough mixing. The pH of the gel (1 g) was determined using a calibrated pH meter. The values were taken as average of 3 reading

3. Results and Discussion

This study evaluated various formulations of minoxidil in situ gels using Gellan Gum and Carbopol as key thickening agents. The analysis focused on multiple parameters: Spreadability, in vitro drug release, clarity, pH, viscosity, and drug content. Understanding these properties is essential for optimizing topical drug delivery systems.

3.1 Evaluation of in-situ gel

3.1.1 Spreadability

Spreadability is an important factor for topical applications, impacting user experience and drug absorption. The formulation with the highest spreadability (F7: 1% Gellan Gum and 0.1% Carbopol) achieved a score of 30.2, indicating a favorable texture that allows for easy application. In contrast, F8 (0.1% Gellan Gum and 1% Carbopol) exhibited the lowest spreadability (22.7), which may be due to the higher Carbopol concentration increasing viscosity, making the gel harder to spread.

3.1.2 *In Vitro* Drug Release

In vitro drug release is critical for assessing the therapeutic efficacy of the formulation. F7 again stood out with the highest release rate (98.5%), indicating that this particular combination of Gellan Gum and Carbopol facilitates optimal drug diffusion. On the other hand, F1 (0.1% Gellan Gum and 0.1% Carbopol) showed the

lowest release (72.4%), likely due to insufficient viscosity and structure to retain and release the drug effectively.

3.1.3 Clarity

All formulations were observed to be clear, a positive characteristic that suggests good formulation stability and homogeneity. Clarity is crucial for consumer acceptance, as a clear gel is typically perceived as more aesthetically pleasing and effective.

3.1.4 pH

The pH of the formulations ranged from 5.7 to 5.9, which aligns well with the skin's natural pH (around 4.5 to 5.5). Maintaining a pH close to physiological levels is important for skin compatibility and minimizing irritation. The consistent pH across the formulations indicates a stable gel environment, which is crucial for both safety and efficacy.

3.1.5 Viscosity

Viscosity measurements varied across the formulations, with values ranging from 9,800 cP (F5) to 14,500 cP (F10). Increased viscosity is generally associated with better retention on the skin but can compromise spreadability. F7 (1% Gellan Gum and 0.1% Carbopol) achieved a good balance, having moderate viscosity (14,000 cP) while maintaining the highest spreadability and drug release. Conversely, F5 had a lower viscosity but also lower drug release, indicating that viscosity needs to be optimized for both performance and user experience.

3.1.6 Drug Content

All formulations demonstrated high drug content, ranging from 97.2% to 99.5%. This suggests that the formulations are effective in encapsulating the active ingredient, which is crucial for therapeutic outcomes. Maintaining a high drug content ensures that the formulation meets the desired dosage requirements.

Formulation code	Factor 1	Factor 2	Response 1	Response 2	Clarity	pH	Viscosity (mPa.s)	Drug Content (%)
	A: Gellan Gum	B: Carbopol	Spreadability (cm)	*In-vitro* Drug Release (%)				
F1	0.1	0.1	25.2	72.4	Clear	5.8	11000	98.2
F2	0.863961	0.55	25.7	94.4	Clear	5.7	11200	98.5
F3	0.55	0.55	25.2	76.8	Clear	5.7	12040	98.2
F4	0.55	0.55	25.2	76.6	Clear	5.7	12040	98.2

F5	1.1864	0.55	28.2	92.5	Clear	5.7	9800	97.2
F6	1	1	25.2	78.7	Clear	5.7	11000	98.2
F7	**1**	**0.1**	**30.2**	**98.5**	**Clear**	**5.7**	**14000**	**99.5**
F8	0.1	1	22.7	79.8	Clear	5.7	14000	97.6
F9	0.55	0.863961	25.7	76.9	Clear	5.9	13200	97.5
F10	0.55	1.1864	24.8	87.2	Clear	5.7	14500	97.9

3.2 Statistical Analysis and Optimization

ANOVA for Linear model

Response 1: Spreadability

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	25.57	2	12.78	7.70	0.0171	significant
A-Gellan Gum	19.83	1	19.83	11.94	0.0106	
B-Carbopol	5.73	1	5.73	3.45	0.1055	
Residual	11.62	7	1.66			
Lack of Fit	11.62	6	1.94			
Pure Error	0.0000	1	0.0000			
Core Total	37.19	9				

- **Final Equation in Terms of Coded Factors**

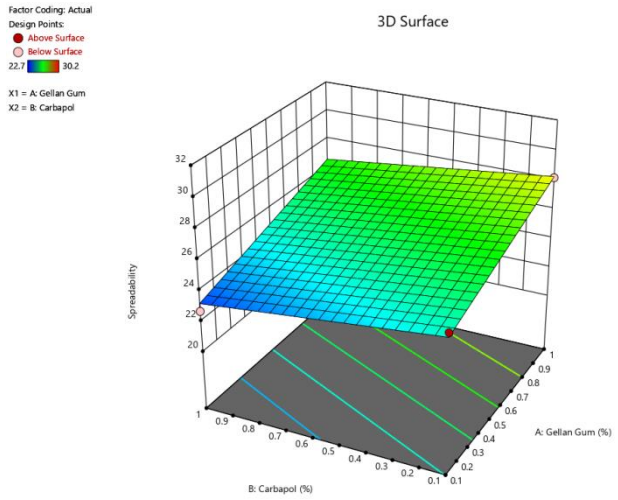
Spreadability = +25.75+ 1.68 A-0.8466 B

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

- **Final Equation in Terms of Actual Factors**

Spreadability = +24.72986+ 3.72809 Gellan gum- 1.88133 Carbopol

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.



ANOVA for Quadratic model

Response 2: *In-vitro* drug release

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	416.13	5	83.23	1.05	0.4964	not significant
A-Gellan Gum	49.38	1	49.38	0.6207	0.4748	
B-Carbopol	8.34	1	8.34	0.1048	0.7624	
AB	112.36	1	112.36	1.41	0.3004	
A^2	139.88	1	139.88	1.76	0.2555	
B^2	2.51	1	2.51	0.0316	0.8676	
Residual	318.23	4	79.56			
Lack of Fit	318.21	3	106.07	5303.46	0.0101	significant
Pure Error	0.0200	1	0.0200			
Core Total	734.36	9				

- **Final Equation in Terms of Coded Factors**

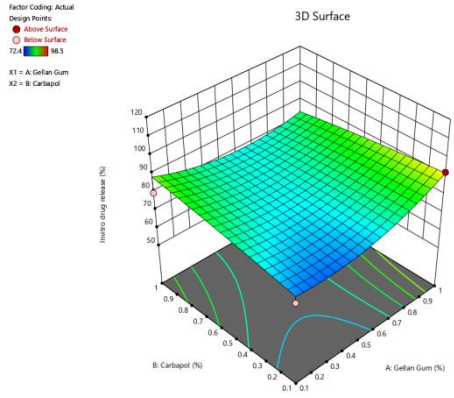
In Vitro drug release= +79.31+2.75A+ 1.02B-5.30AB+6.43 A^2-0.7135 B^2

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

- **Final Equation in Terms of Actual Factors**

In Vitro drug release= +75.31148-1439831 Gellan gum+20.53910 Carbapol-26.17284 Gellan gum*Carbapol+31.73834 Gellan gum^2-3.52327 Carbopol

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space



Constraints

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A:Gellan Gum	is in range	0.1	1	1	1	3
B:Carbapol	is in range	0.1	1	1	1	3
Spreadability	maximize	22.7	30.2	1	1	3
Invitro drug release	maximize	72.4	98.5	1	1	3

Solutions

Number	Gellan Gum	Carbopol	Spreadability	*In-vitro* drug release	Desirability	
1	**1.000**	**0.100**	**28.270**	**92.053**	**0.748**	**Selected**

2	1.000	0.107	28.257	92.010	0.746	
3	0.992	0.100	28.239	91.667	0.738	
4	0.982	0.100	28.203	91.232	0.728	
5	0.969	0.100	28.156	90.663	0.713	
6	1.000	0.250	27.988	91.023	0.709	
7	1.000	0.599	27.330	88.008	0.608	

Factors

Factor	Name	Level	Low Level	High Level	Coding
A	Gellan Gum	1.0000	0.1000	1.0000	Actual
B	Carbopol	0.1000	0.1000	1.0000	Actual

Point Prediction

Two-sided Confidence = 95% Population = 99%

Solution 1 of 7 Response	Predicted Mean	Predicted Median	Std. Dev.	SE Mean	95% CI low for Mean	95% CI high for Mean	95% TI low for 99% Pop	95% TI high for 99% Pop
Spreadability	28.2698	28.2698	1.28855	0.769064	26.4513	30.0884	20.8479	35.6918
In-vitro drug release	92.0529	92.0529	8.91947	7.04875	72.4824	111.623	22.5211	161.585

Confirmation Location #1

Gellan Gum	Carbopol
1	0.1

Confirmation

Two-sided Confidence = 95%

Solution 1 of 7 Response	Predicted Mean	Predicted Median	SE Mean	95% CI low for Mean	95% CI high for Mean	95% TI low for 99% Pop	95% TI high for 99% Pop
Spreadability	28.2698	28.2698	1.28855	3	1.07001	25.7397	27.6667

In-vitro drug release	92.0529	92.0529	8.91947	3	8.72948	67.816	91

Factors

Factor	Name	Level	Low Level	High Level	Std. Dev.	Coding
A	Gellan Gum	1.0000	0.1000	1.0000	0.0000	Actual
B	Carbopol	0.1000	0.1000	1.0000	0.0000	Actual

4. Conclusion

The evaluation of the *in situ* gel formulations of minoxidil highlights the importance of balancing Gellan Gum and Carbopol concentrations to optimize spreadability, drug release, and viscosity. Formulation F7, with its favorable properties, stands out as the most promising candidate for further development. Future studies could focus on stability testing and clinical evaluations to confirm the efficacy of this formulation in real-world applications. Additionally, exploring other combinations of excipients may yield even more effective formulations.

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