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Nanogel-Based Polyherbal Approach for Hyperpigmentation: Harnessing the Therapeutic Potential of Azadirachta Indica, Allium Cepa, and Curcuma Longa

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ABSTRACT

Nanogels are a type of nanostructured polymeric material that can be used for delivering therapeutic agents, especially in areas like drug delivery, cosmetics, and dermatology. Hyperpigmentation is a condition where certain areas of the skin become darker than the surrounding skin due to an excess production of melanin, the pigment responsible for skin color. Nanogels can protect curcumin from oxidation and ensure its prolonged release to maximize its skin-brightening benefits. The aim of the current work is to formulate polyherbal nanogel utilizing herbal ingredients (*A.indica, Curcuma longa* and *allium cepa*) can be encapsulated within the gel's network, ensuring that the active compounds are delivered directly to the site of pigmentation with the help of Central Composite design and evaluated for particle size, entrapment efficiency, surface morphology and *in-vitro* drug release. Thirteen formulations were prepared using design expert software with two independent variables i.e., concentration of Pluronic F127 and concentration of polyethyleneimine at two different levels. Based on the desirability function, optimized batch was further characterized for particle size, surface morphology and *in-vitro* studies. The results of optimized formulation with reference to particle size, entrapment efficiency and in-vitro study were 114.7nm, 82.41% and 94.588±0.008%. The purpose of the research work was to provide controlled release of the compounds from nanogels will allow for prolonged therapeutic effects, ensuring that the ingredients penetrate the skin effectively and work continuously over time. Future research should focus on optimizing the formulation, evaluating its clinical efficacy through in vivo studies, and assessing its long-term safety and effectiveness.

Keywords: *Nanogel, Cental Composite Design, Azadirachta indica, curcuma longa, allium cepa, Hyperpigmentation.*

1. INTRODUCTION

Nanogels are a type of nanostructured polymeric material that can be used for delivering therapeutic agents, especially in areas like drug delivery, cosmetics, and dermatology. They are made up of hydrophilic (water-loving) networks of nanometer-sized (usually 1-1000 nm) polymers that may swell in water and encapsulate a range of bioactive substances. Nanogels are a flexible and efficient method for targeted delivery since they may be used to encapsulate medications or active substances. Three steps were used to prepare the nanogel: 1,1-carbonyl diimidazole-induced Pluronic F127 activation; F127/PEI nanogel preparation; drug-loaded F127/PEI nanogel. The F127/PEI nanoparticles was prepared by solvent evaporation method. Pluronic F127 is well-known for its capacity to self-assemble into a gel-like structure in aqueous conditions and to create hydrogels at particular temperatures. [1] A polymeric cationic substance called polyethyleneimine is frequently added to nanogel formulations as a stabiliser and to improve the effectiveness of drug encapsulation. For improved treatment of hyperpigmented regions, it increases medication loading capacity, improves skin adherence, and increases the encapsulation effectiveness of active substances (such as curcumin and quercetin). To stabilise the nanogel and provide regulated release of active ingredients for an extended course of therapy for hyperpigmentation, carbonyl diimidazole (CDI) cross-links the polymer network. [2,3]

The distinct qualities of neem (*Azadirachta indica*), onions (*Allium cepa*), and turmeric (*Curcuma longa*) can all be very helpful in the treatment of hyperpigmentation. [4] These three natural substances include active chemicals that can help address many pathways that cause hyperpigmentation, including inflammation, oxidative stress, and excess melanin synthesis.

Neem (*Azadirachta indica*) aids in skin regeneration, irritation relief, and a decrease in melanin synthesis. [5.6] Quercetin, found in onions (*Allium cepa*), has anti-inflammatory and skin-lightening properties. For long-lasting anti-pigmentation benefits, quercetin may be released gradually via the nanogel. *Curcuma longa*, or turmeric, brightens the skin tone, prevents the production of melanin, and offers antioxidant protection. To optimise curcumin's skin-brightening properties, nanogels can shield it from oxidation and guarantee its sustained release. [7,8]

A condition known as hyperpigmentation occurs when the pigment melanin, which gives skin its colour, is produced in excess in some parts of the skin, making those regions darker than the rest of the skin. Numerous internal and environmental variables, such as hormone fluctuations, inflammation, trauma, acne, eczema, certain medications, UV exposure, etc., can cause these colour changes in the skin. Spots or patches of skin that are darker than the rest of the skin may be the result of this extra melanin. [9] The biological mechanisms involving the synthesis of melanin, the skin pigment, by melanocytes in the different layers of skin control skin pigmentation and colouration. Therefore, skin hyperpigmentation diseases are caused by changes in melanocyte production or melanin dispersion. Melasma, post-inflammatory hyperpigmentation, ephelides, lentigines, and many more are examples of hyperpigmentation diseases that are frequently seen. Melasma is a skin disorder known as acquired hypermelanosis, in which sun-exposed areas of the skin develop uneven patches of light to dark brown or gray-brown lesions. It is primarily seen in women and typically affects the face and neck areas. Another hypermelanosis skin disease known as post-inflammatory hyperpigmentation (PIH) occurs when dark areas appear after skin inflammation or damage. Solar lentigines, often known as "Age spots" or "Sunspots," is a disorder in which areas of darkened macular lesions result in hyperpigmentation. Another prevalent condition is Ephelides, often known as freckles, which are pigmented, reddish to light brown patches that usually appear on the arms, neck, and face.[10]

The aim of the current work is to formulate polyherbal nanogel utilizing herbal ingredients (*A.indica*, *Curcuma longa* and *allium cepa*) can be encapsulated within the gel's network, ensuring that the active compounds are delivered directly to the site of pigmentation with the help of Central Composite design and evaluated for particle size, entrapment efficiency, surface morphology and *in-vitro* drug release. The purpose of the research work was to provide controlled release of the compounds from nanogels will allow for prolonged therapeutic effects, ensuring that the ingredients penetrate the skin effectively and work continuously over time.

2. MATERIALS AND METHODS

2.1 Materials

Azadirachta indica, *curcuma longa* and *allium cepa* extract was obtained and authenticated from Green Vibes Biotech, New Delhi. All other reagents used were of analytical grade. Pluronic F127, Polyethyleneimine and Carbonyl Diimidazole were purchased from CDH, New Delhi.

2.2 Methods

2.2.1 Optimization of Polyherbal Nanoparticles using Central Composite Design

The formulation of optimised polyherbal nanoparticles was prepared using the two factor and two level central composite design (CCD). The design is based on the response surface method (RSM). The concentrations of polyethyleneimine (X2) and pluronic F127 (X1) were selected as independent variables. As indicated in Table 1, the software has provided two axial points for each independent variable in addition to low (-1) and high (+1). Particle size (nm) and entrapment efficiency (%) were the dependent variables. The design expert program provided a total of thirteen formulations. [11]

Table 1: Independent Variables and Their Levels in CCD

Variable	**Level**	
Independent variables	**Low (-1)**	**High (+1)**
X1: Concentration of Pluronic F127 (mg)	100	300
X2: Concentration of Polyethyleneimine (PEI) (mg)	50	250
Coded Values	-1	+1
Dependent variables		
Y1=particle size (nm)		
Y2=Encapsulation efficiency (EE) (%)		

2.2.2 Preparation of Nanoparticles using Central Composite Design

Three steps were taken to prepare the nanogel: Pluronic F127 is activated by 1,1-carbonyl diimidazole; F127/PEI nanogel is prepared; and F127/PEI nanogel is drug-loaded. An excess of CDI in THF was mixed with a dropwise addition of Pluronic F127 in anhydrous THF. To obtain CDI-activated Pluronic F127, the solution was filtered out after being concentrated to a small volume under vacuum and then poured into ethyl ether. To get rid of the unreacted CDI, this procedure was carried out three times. After 12 hours of vacuum-assisted room temperature drying, the CDI-activated Pluronic F127 was produced as a white powder. Solvent evaporation was used to create the F127/PEI nanoparticles. After being dissolved in chloroform, the activated Pluronic F127 was gradually added to an aqueous PEI solution while being stirred. The organic solvent in the emulsion was eliminated by rotational vacuum evaporation at 50°C for 45 minutes after the mixture had been sonicated for three minutes.

Table 2: Formulation Table obtained by software

Formulation	Concentration of Pluronic F127 (mg)	Concentration of Polyethyleneimine (PEI) (mg)	Drug Combination (w/w)
F1	200	150	1%
F2	200	150	1%
F3	200	150	1%
F4	200	8.57864	1%
F5	200	150	1%
F6	300	50	1%
F7	100	250	1%
F8	200	291.421	1%
F9	100	50	1%
F10	300	250	1%
F11	200	150	1%
F12	58.5786	150	1%
F13	341.421	150	1%

2.2.3 Formulation of optimized batch of polyherbal Nanogel

Curcuma longa, allium cepa, and azadirachta indica extract powder (1% w/w) and lyophilised empty nanogels were dissolved separately in a 1:1 methanol and water mixture, then combined. The solvent was then eliminated using rotary vacuum evaporation. Phosphate buffered saline pH 7.4 was added in an appropriate quantity to further hydrate the resultant film.

3. EVALUATION OF NANOPARTICLES

3.1 Particle Size Determination

Nanoparticles formulation was diluted with water, for particle size determination using dynamic light scattering analyser (DLS). [12]

3.2 Entrapment Efficiency

The formulations were dissolved in a minimum quantity of methanol individually and centrifuged at 1,500 rpm for 20 minutes. The sediments were separated and upper layers were filtered, suitably diluted and analyzed spectrophotometrically at respective wavelengths. Each experiment was repeated in triplicate. [12] Percentage drug entrapment, for each class of nanoparticles, was determined by the following formula:

$$\text{E. E.} = \frac{\text{Amount of drug actually present in nanoparticles} \times 100}{\text{Amount of drug actually used}}$$

3.3 In-vitro Drug Release Study

Franz diffusion cells and dialysis membrane 50 were used in an in vitro drug release evaluation of the produced nanogels. 25 millilitres of phosphate buffered saline pH 7.4 diffusion media were placed inside the receptor compartment. The donor compartment was positioned so that it only comes into contact with the receptor compartment's diffusion medium. The donor chamber was filled with 5 millilitres of nanogel. Throughout the study, the vessels were double-jacketed with water flowing between the jacket walls to maintain the temperature at 37 ± 0.5°C. The entire assembly was fixed on a magnetic stirrer, and the solution in the receptor compartment was continuously stirred using magnetic beads to ensure uniform distribution of permeating solutes for later sampling. 2 ml sample of the receptor fluid were withdrawn at predetermined time intervals and replaced immediately with same volume of fresh diffusion media.

4. RESULTS AND DISCUSSION

The solvent evaporation process was used to create the nanogel. *Curcuma longa*, *allium cepa*, and *azadirachta indica* extract powder (1% w/w) and lyophilised empty nanogels were dissolved separately in a 1:1 methanol and water mixture, then combined. The solvent was then eliminated using rotary vacuum evaporation. Phosphate buffered saline pH 7.4 was added in an appropriate quantity to further hydrate the resultant film.

4.1 Central Composite Design for the optimization of Nanoparticles

Thirteen formulations in all were created and assessed for entrapment efficiency (Y2) and particle size (Y1). Design expert software was used to determine the values of each parameter. ANOVA was used to statistically assess each response in accordance with the design. When made fresh, all of the formulations looked like opaque, dull yellow mixes without any particles visible. The entrapment efficiency was determined to be between 69.2-86.1%, while the resulting particle size of all thirteen formulations was between 110.7-295.1 nm.
As shown in Table 5, the best fit polynomial model for both responses—particle size (Y1) and entrapment efficiency (Y2)—was determined to be linear (p value < 0.005). [13]

4.1.1 Effect of Independent factors on particle size

The different combinations of Pluronic F127 (mg) (X1) and polyethyleneimine (mg) (X2), for the Y1 (particle size) response gave a mathematical association in the form of linear equation as shown below:

Particle Size (Y1): 199.72+ 26.91 X1 + 58.31 X2…… Coded Equation 1

As shown in Table 3, the value of p (0.05) of the coefficients indicates the statistical significance of the response. The sign and numerical value of the coefficients provide an instantaneous comprehension of the form and extent of the influence on responses. If the independent components' coefficients are negative, the influence will be opposing; if they are positive, it will be beneficial. A considerable influence on the reaction is indicated by a larger factor value. The design was analysed using a linear model. The model's effectiveness was confirmed by the ANOVA and the multiple correlation analysis (R2). The p-value of 0.0017 and the R2 value of 0.7208, as shown in Table 3, demonstrate the statistical significance of independent components in response. In Figure 1(A), (B), and (C), the particle size findings are combined as response plots, contour plots, and predicted vs. actual plots, correspondingly. Also, there is a less than 0.2 variation between the adjusted R2 (0.6649) versus the predicted R2 (0.4947) demonstrating the model's good fit. This model's F value was determined to be 12.91.

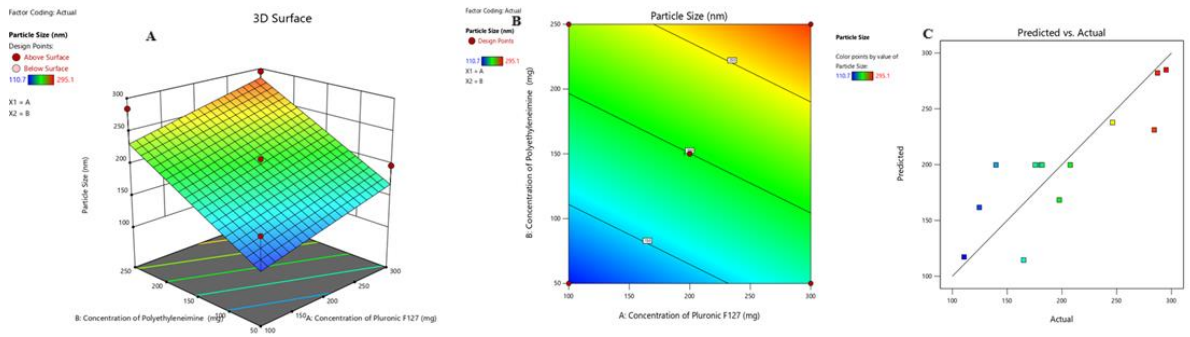

Figure 1: Effect of independent variables on Particle size (A) Response plot (B) Contour Plot (C) Predicted vs Actual Plot

4.1.2 Effect of Independent Variable on Entrapment Efficiency

As per the results, the entrapment efficiency (Y2) of the polyherbal nanoparticles ranged between 69.2-86.1%. The linear equation obtained is:

Entrapment Efficiency (Y2): 76.41 + (-1.88 X1) + (-4.79 X2)……. Coded equation 2 The above equation shows the

negative coefficients of X1 and X3 representing the inverse effect of variables on response. As the concentration of Pluronic F127 and PEI increases, the entrapment efficiency decreases. The p value (< 0.05) of this model displays significance of the model. The results are compiled in the form of response plot, contour plot, and predicted vs actual response in Figure 2 (A), (B) and (C) respectively.

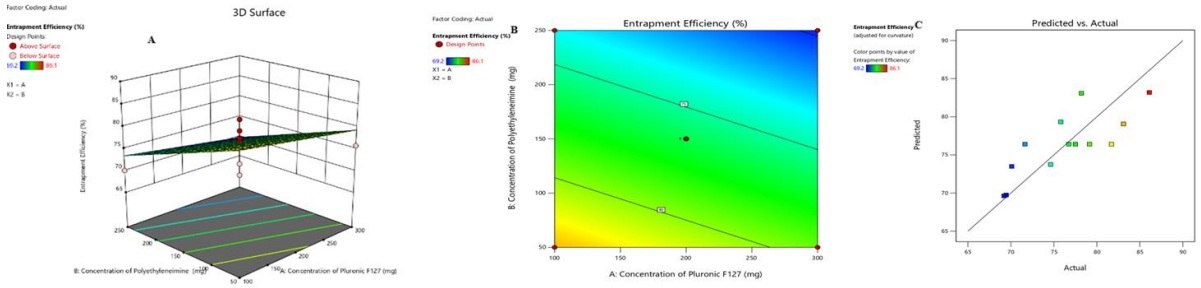

Figure 2: Effect of independent variable on Entrapment Efficiency (A) Response Plot (B) Contour plot (C) Predicted vs Actual Plot

Table 3: ANOVA of the responses and their model fitting statistics

Response	Model Fitting	R2	Adjusted R2	Predicted R2	SD	Model F-value	Model p-value	% CV
Y1	Linear	0.7208	0.6649	0.4947	35.75	12.91	0.0017	
Y2	Linear	0.6144	0.5373	0.3272	3.65	7.97	0.0085	4.77

Table 4: Coefficients of Independent factors and their related probability (p) value

	Intercept	A	B
Particle Size	199.723	26.908	58.306
p-values		0.0591	0.001
Entrapment Efficiency	76.4085	-1.878	-4.793
p-values		0.176	0.004

4.2 Development of Optimized Formulation

The optimal concentration of the independent variables that can accommodate the constraints—minimum particle size and maximum entrapment efficiency—was identified by the dependent variables' results. According to the design, the concentrations of polyethyleneimine and pluronic F127 should be 50 and 100 mg, respectively.

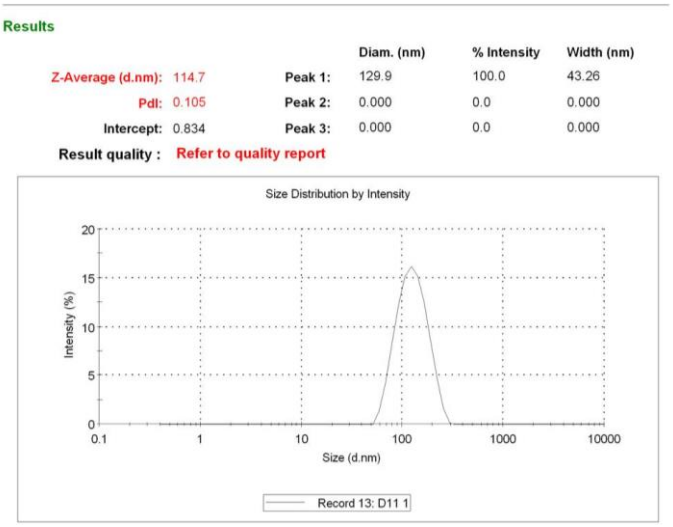

Figure 3: Particle Size of optimized batch

Surface Morphology

The optimized formulation was found to be 114 nm with rough surface as shown in figure 7.

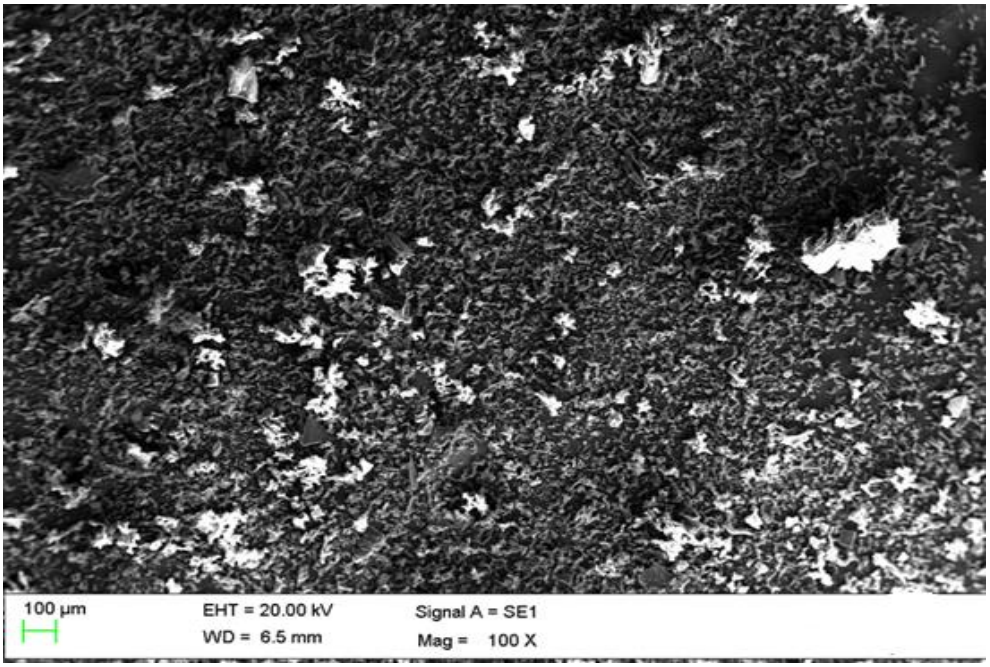

Figure 4: SEM image of optimized batch

***In-vitro* Drug Release Studies**

The *in-vitro* drug release of the optimized formulation was performed in PBS at pH 7.4. The cumulative percentage drug release was found to be 83.39% ± 1.96 at the end of 12 h as shown in Figure 8. The graph shows a bi-phasic pattern release. Initially, burst release was observed of 70.47% ± 0.007 up to 6h, then the release become sustained upto 24 h. The former might be due to surface adsorption of herbal ingredients on nanoparticulate surface, while the latter may be attributed to the lipid-matrix formation in which drug is entrapped. [14]

Table 5: *In-vitro* drug release of optimized batch

Time (Hr)	PHN-1
0.5	15.06±0.004
1	27.14±0.015
2	38.16±0.006
3	49.03±0.019
4	59.87±0.022
6	70.47±0.007
8	79.33±0.026
10	88.65±0.015
12	94.588±0.008

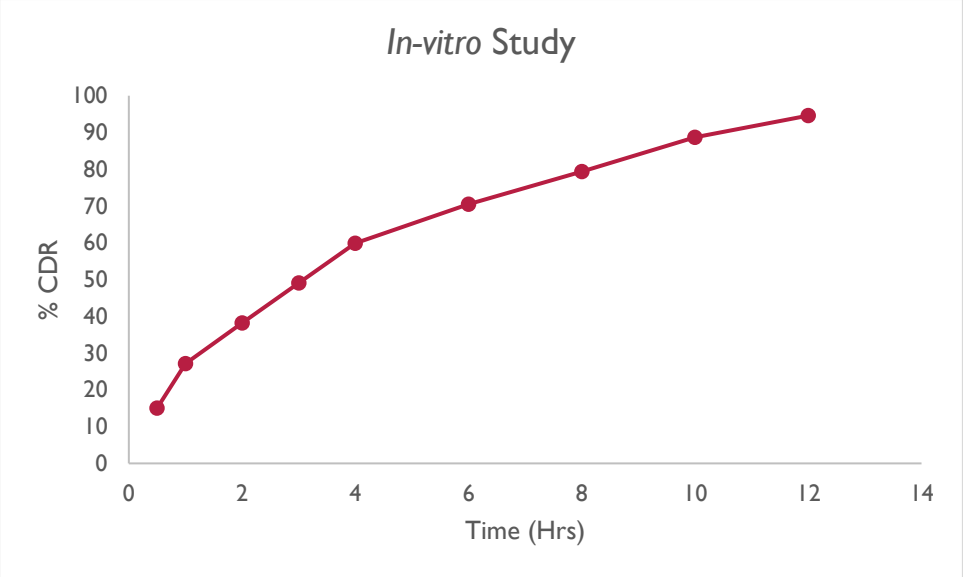

Figure 5: *In-vitro* drug release study of optimized polyherbal nanoparticles formulation

5. CONCLUSION

The potential of a nanogel-based polyherbal treatment for hyperpigmentation was investigated in this work by utilising the complementary qualities of Curcuma longa (turmeric), Allium cepa (onion), and Azadirachta indica (neem). By focussing on important processes including melanin synthesis, skin irritation, and oxidative stress, the active compounds in neem, onion, and turmeric provide a comprehensive approach to treating hyperpigmentation. Together, the anti-inflammatory and melanin-regulating qualities of neem, the antioxidant and skin-brightening qualities of turmeric, and the ability of onions to suppress melanin synthesis and encourage collagen creation help to lessen black spots, balance skin tone, and encourage skin regeneration. These substances have a long-lasting therapeutic impact because they are shielded from deterioration by the nanogel.

In conclusion, the nanogel-based polyherbal approach holds significant promise as an innovative and effective solution for hyperpigmentation treatment. Future research should focus on optimizing the formulation, evaluating its clinical efficacy through in vivo studies, and assessing its long-term safety and effectiveness. This approach not only opens new avenues in cosmetic dermatology but also highlights the potential of combining natural plant-based therapies with advanced nanotechnology to address skin disorders in a holistic and sustainable manner.

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