



Spirulina Based Zinc Oxide Nanoparticle Microgel Effective Against Acne

Ajay S. Dongarwar*, Aman A. Khelkar¹, Anand D. Ambule², Shubham R. Uikey³, Switil K. Chaure⁴, Vicky Y. Bhowte⁵

Department of Pharmaceutics, Manoharbhair Patel Institute of Pharmacy (B.Pharm) Kudwa, Gondia, MH-441614 INDIA

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ABSTRACT

The study of novel ecofriendly and cost effective approach for a synthesis of zinc oxide nanoparticle using spirulina extract and their formulation used for cosmetic purpose. A nanoparticle is an extremely small particle with dimensions ranging from one to one hundred nanoparticle. Nanoparticle is used in drug delivery system and its synthesis with green chemistry has gained prominence in numerous research fields for its role in enhancing and protecting human health. The pharmaceutical application of nanoparticle present significant potential for developing new biomedical science. The spirulina medicated zinc oxide nanoparticle microgel provide sustainable and environmental ecofriendly approach for the production of formulation in cosmeceuticals. Spirulina used as biogenic agent, it is a cyanobacterium, rich source of nutrients including proteins, vitamins and minerals. It have unique immunomodulatory, antioxidant, antimicrobial, wound healing properties. Spirulina ability to synthesize zinc oxide nanoparticle can attributes to its unique biochemical composition. The nanoparticle were characterised by using UV spectroscopy and DSC (Differential Scanning Calorimetry). Microgel is a semisolid material consist of network of polymer chain its swollen with a solvent typically a liquid. The evaluation of microgel by antimicrobial studies against gram positive and gram negative bacteria and formulate effective microgel using in cosmetic purpose.

Keywords:- Spirulina extract, biogenic synthesis, zinc oxide nanoparticle, microgel, cosmetics, biomedical application, antimicrobial activity.

1. INTRODUCTION

Nanoparticles are tiny particles with unique properties that make them useful for a wide range of applications. The synthesis of nanoparticles, particularly zinc oxide nanoparticles (ZnO NPs), has gained significant attention due to their unique properties. Nanoparticles are playing an increasingly important role in pharmaceuticals. Spirulina are multicellular and filamentous blue-green algae that has gained considerable popularity in the health food industry and increasingly as a protein and vitamin supplement to aquaculture diets. It grows in water, can be harvested and processed easily and has very high macro- and micro-nutrient contents. Spirulina that belongs to the genus *Arthrospira*. It is a cyanobacterium, a single-celled microorganism that obtains its energy through photosynthesis, like plants. Spirulina is considered one of the most nutritious and oldest living organisms. Algae are photosynthetic organisms that convert light energy from the sun into the chemical energy by the process of photosynthesis. Algae possess simple reproductive structure. The biomass of algae contains various compounds with diversified structures and functions.

Nanotechnology

Nanotechnology is a promising technique for the development of new products because it can improve the nutritional value and protection of active compound without changing the physical properties. A nanoparticle is an extremely small particle with dimensions ranging from one to one hundred nanometers.

Microgel

Microgel is a semisolid material consist of network of polymer chain its swollen with a solvent typically a liquid. The evaluation of microgel by antimicrobial studies against gram positive and gram negative bacteria and formulate effective microgel using in cosmetic purpose. Microgels will be defined as crosslinked polymers roughly of spherical structure with dimensions of the order of the size of ordinary polymer molecules, branched polymer or lattice animals. The formulation are commonly used in topical application like gel, cream, lotion, ointment and their evaluation are influenced by factor like appearance, spreadability, viscosity and pH.



Fig 1 :- Microgel

2. MATERIALS AND METHODS

2.1 Materials For Formulation

Ingredient :- Extract of spirulina, Zinc acetate, Zinc oxide, Methanol, Carbapol 934, Acacia gum, Methyl paraben, Sodium hydroxide, Propylene glycol, Glycerin, Rose water, Citric acid, Distilled Water.

Equipment :- Digital balance, PH meter, Autoclave, Hot air oven, B.O.D Incubator, Heating mantle, UV Visible spectrophotometer, DSC (differential scanning calorimetry), Measuring cylinder, Mechanical stirrer, Centrifuge, Magnetic stirrer, Thermometer, Conical flask, Soxhlet apparatus.

2.2 Experimental Work

Preparation of Spirulina extract

Solvent extraction:- This method involve using a solvent such as ethanol and methanol to extract bioactive compound from spirulina.

Procedure :-

1. Place a solid sample in a thimble-shaped filter paper.
2. Position the filter paper in a Soxhlet extractor.
3. The extractor uses solvent reflux and siphon to continuously extract the solid matter.



Fig 2:- Soxhlet Assembly



Synthesis of zinc oxide nanoparticle by Spirulina extract

1. Prepare the solution: Stir 50 mL of 10 mM $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2.2\text{H}_2\text{O}$ solution for 30 minutes.
2. Add the extract: Dropwise add 10 mL of a freshly prepared methanol extract of *Spirulina platensis* (1% w/v).
3. Stir and form the precipitate: Stir at 800 rpm for 3-4 hours at 50 °C until a cream-colored zinc hydroxide precipitate forms.
4. Reduce the zinc hydroxide: Let the solution sit for 30 minutes.
5. Centrifuge: Centrifuge the reaction mixture at 4100 rpm for 12 minutes.
6. Collect and wash: Collect the whitish sediments and wash with distilled water.
7. Calcine: Calcine the ZnO NPs mixture for 4 hours at 455 °C in a muffle furnace to obtain pure ZnO NPs.



Fig 3:- Centrifugation of mixture

Formulation of microgel

Ingredients

1. Gelling Agent: 1 gm Carbapol 934
2. Solvent: 100 ml water
3. Active Ingredient: 0.3 gm spirulina with zinc oxide nanoparticle
4. Thickener: 5 g acacia gum (powder)
5. Preservative: 0.1gm methyl paraben , 0.2 gm propyl paraben
6. pH Adjuster: 0.5 g citric acid (powder)

Procedure

1. Weighing and Dispensing: Accurately weigh and dispense the ingredients
2. Mixing: Combine the gelling agent, solvent, and active ingredient in a specific order.
3. Heating/Cooling: Apply heat or cooling to facilitate dissolution, gelation, or other processes.



4. Homogenization: Ensure uniformity of the gel using techniques like stirring, shaking, or homogenization.
5. Thickening: Add thickeners to adjust the gel's viscosity.
6. Preservation: Add preservatives to prevent microbial growth.
7. pH Adjustment: Adjust the pH using pH adjusters.
8. Filling and Packaging: Fill the gel into suitable containers.

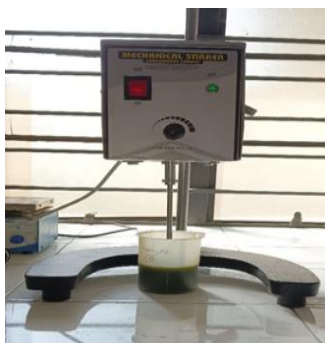


Fig 4 :- Homogenization

2.3 Evaluation Of Formulation

Phytochemical analysis of spirulina extract

Test for Alkaloids

2 mL of concentrated Hydrochloric acid (HCl) was added to 2 mL Spirulina platensis extract. Then few drops Mayer's reagent was added. Presence of green colour or white precipitate indicates the presence of alkaloids.

Test for Tannins

1 mL of ferric chloride (5% FeCl₃) was added to 1 mL of the Spirulina platensis extract. Formation of dark blue or greenish black colour indicates the presence of tannins.

Test for Saponins

2 mL of distilled water was added to 2 mL Spirulina platensis extract and shaken in graduated cylinder for 15 min lengthwise. Formation of 1 cm layer of foam indicates the presence of saponins.

Test for Flavonoids

1 mL of 2N sodium hydroxide (NaOH) was added to 2 mL of Spirulina platensis extract. Formation of yellow colour indicates the presence of flavonoids.

TLC (Thin Layer Chromatography)

TLC is a widely used analytical technique for separating, identifying, and quantifying the components of a mixture. In the case of Spirulina extract, TLC can be used to separate and identify the various bioactive compounds present in the extract.

Evaluation of Nanoparticle

1. UV- Visible Spectroscopy



UV-Visible absorption spectra: The UV-Visible absorption spectra of ZnO NPs were recorded using a UV-Visible spectrophotometer. Wavelength range: The wavelength range was set from 200 to 800 nm.

2. DSC (Differential Scanning Calorimetry)

DSC (Differential Scanning Calorimetry) is a thermal analysis technique used to measure the heat flow into or out of a sample as it is heated or cooled. Melting point: MP of sample range between 200 to 300 (264.3 celcius).

Evaluation Of Microgel Formulation

Formulation was tested for physical appearance, colour, odour, homogeneity, spreadability, washability, skin irritation.

Anti Microbial study

Determination of minimum inhibitory concentration (MIC): To determine the MIC of ZnO NPs against various microorganisms. Assessment of antimicrobial mechanism: To assess the antimicrobial mechanism of ZnO NPs synthesized using Spirulina extract.⁽²²⁾

Microorganisms

1. Gram-positive bacteria: Staphylococcus aureus, Bacillus subtilis
2. Gram-negative bacteria: Escherichia coli, Pseudomonas aeruginosa

Staphylococcus aureus

The invitro antibacterial activity of zinc oxide nanoparticle synthesis by spirulina extract against isolated colonies by staphylococcus aureus investigated using agar medium to check the zone of inhibition of bacterial colonies.

3. RESULT AND DISCUSSION

3. 1 Physical Characteristics Of Nanoparticle

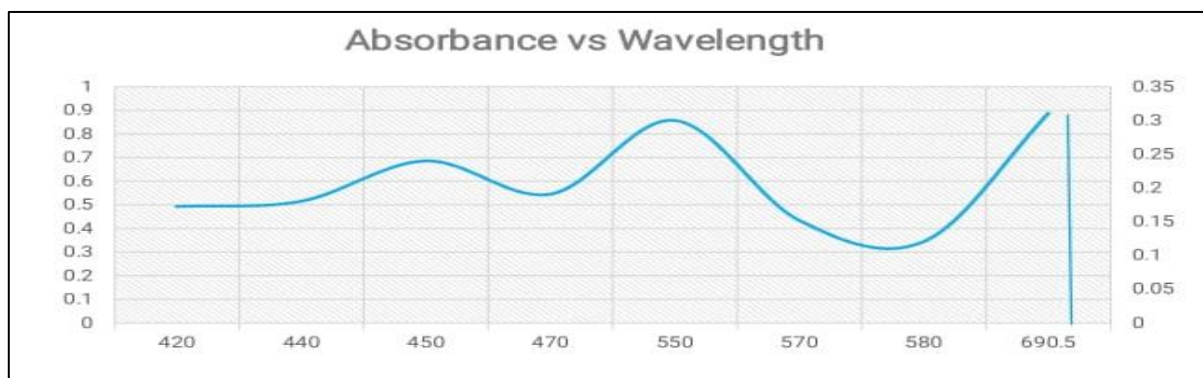
Table 1 :- Physical Characteristics

Colour	Bluish green
Odour	Algal type
Temperature	38
Melting point	295

UV Visible Spectroscopy

Table 2 :- Absorbance vs Wavelength of ZnoNPs

Absorbance	Wavelength
0.170	420
0.180	440
0.240	450
0.150	570
0.120	580
0.308	690
0.266	800



The UV spectroscopy of ZnO NPs synthesized using Spirulina extract showed a strong absorption peak at around 308 nm.

DSC (Differential Scanning Calorimetry)

Melting point: MP of sample range between 200 to 300 cel. (264.3 cel)

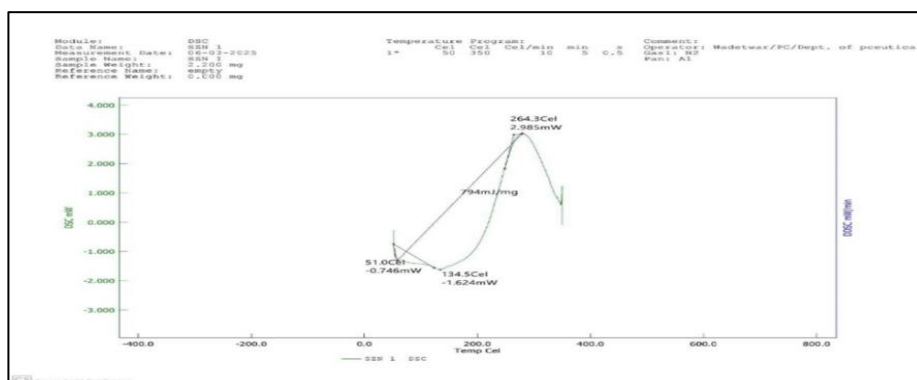


Fig 5 :- DSC analysis of zinc oxide of nanoparticle using spirulina extract

3.2 Result of evaluation parameter of microgel

Table 3 :- Result of evaluation parameter

Sr. No.	Parameter	Day 1	Observation F2 Formulation Day 3	Day 7
1	Colour	Greenish	Greenish	Greenish
2	Odour	Characteristics	Characteristics	Characteristics
3	Nature	Semisolid	Semisolid	Semisolid
4	Consistency	Smooth	Smooth	Smooth
5	Homogeneity	Homogenous	Homogenous	Homogenous
6	Washability	Washable	Washable	Washable
7	PH	9.49	9.47	9.36
8	Skin irritation	No irritant	No irritant	No irritant
9	Patch test	No allergic	No allergic	No allergic
10	Temperature	26	27	25
11	Viscosity	600cp	655cp	650cp
12	Spreadability	5.50 cm/sec	5.60 cm/sec	5.46 cm/sec
13	Microbial	antimicrobial	antimicrobial	antimicrobial



Formulation F1, F2, F3 was tested using various evaluation parameters. Spreadability, viscosity and pH of F2 formulation was found very good when compared to F1 and F3.

Stability studies: stability studies of F2 formulation gives good results during 7 days and the values are below.

Table 4 :- Result Of Stability Studies

Sr. No.	Parameter	F1	Observation F2	F3
1	Colour	Greenish	Greenish	Greenish
2	Odour	Characteristics	Characteristics	Characteristics
3	Nature	Semisolid	Semisolid	Semisolid
4	Consistency	Smooth	Smooth	Smooth
5	Homogeneity	Homogenous	Homogenous	Homogenous
6	Washability	Washable	Washable	Washable
7	PH	9.49	9.47	9.36
8	Temperature	26	27	25
9	Viscosity	505cp	655cp	705cp
10	Spreadability	5.70 cm/sec	5.60 cm/sec	5.66 cm/sec
11	Microbial	antimicrobial	antimicrobial	antimicrobial

F2 more effective than F1 and F3. We can use this microgel for getting best results for acne treatment.

Anti Microbial Study

Table 5 :- Zone of inhibition of Erythromycin and ZnONPs against S. aureus

Sr. No	Sample	Zone Of Inhibition		
		A	B	C
1	Erythromycin	0.8mm	1.4mm	1.9mm
2	Sample	1.6mm	2.2mm	2.5mm

Table 6 :- Zone of inhibition of Erythromycin and sample against Escherichia coli

Sr. No	Sample	Zone Of Inhibition		
		A	B	C
1	Erythromycin	0.7mm	1.6mm	2.4mm
2	Sample	1.6mm	2.2mm	3.2mm

4. DISCUSSION

The biogenesis synthesis of spirulina extract using zinc oxide nanoparticles has shown promising results. The addition of zinc oxide nanoparticles to the culture medium enhances the growth and biochemical composition of spirulina. The antioxidant activity of the spirulina extract also increases with the addition of zinc oxide nanoparticles.

5. CONCLUSION

The present study investigate the synthesis of Spirulina extract loaded zinc oxide nanoparticles microgel as a novel topical treatment for acne. The results show that the microgel formulation exhibits excellent antimicrobial activity against reduces inflammation and promotes wound healing. The Spirulina extract loaded zinc oxide nanoparticles microgel offers a promising novel treatment for acne, with its antimicrobial, anti-inflammatory, and wound healing promoting effects. Further studies are needed to optimize the formulation and evaluate its clinical efficacy. The charecterisation of nanoparticle by UV Spectroscopy and DHC study. The microgel formulation and evaluation is effectively synthesise to enhance antimicrobial activity and prevent the acne.



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