RESEARCH PAPER

Formulation of Peel-Off Gel Mask from Reduced Graphene Oxide and Chitosan by Box-Behnken Design: Evaluation of Antibacterial and Antifungal Activity

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ABSTRACT

The aim of this study was to develop a peel-off gel mask with potential antimicrobial properties using reduced graphene oxide (rGO) derived from rice husk and chitosan. rGO was synthesized through a green method using lemon juice as a reducing agent, and its properties were optimized using a two-level factorial design. Chitosan-based gel formulations were prepared using a Box–Behnken design, considering polymer concentration, glycerol content, and ethanol volume as independent variables. The formulations were evaluated for drying time, film thickness, gel dispersion, and folding endurance. The optimized rGO–chitosan gel showed significant antibacterial activity against Escherichia coli and Staphylococcus aureus, along with antifungal activity against Candida albicans. Characterization techniques including scanning electron microscopy, FT-IR, EDAX, and zeta potential analysis confirmed successful incorporation of rGO into the polymer matrix. These findings suggest that the developed peel-off gel mask offers a promising natural alternative for acne treatment with effective antimicrobial activity and desirable formulation properties.

Keywords: Anti-microbial activity, Box-Behnken design, Chitosan, Peel-off gel mask, Reduced graphene oxide

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Introduction

Acne vulgaris is a common dermatological condition affecting approximately 80% of adolescents and young adults, often resulting in physical scarring and psychological distress due to associated anxiety and embarrassment[1]. Conventional treatments, including topical and systemic antibiotics, are limited by adverse effects and the emergence of antibiotic-resistant pathogens, highlighting the need for safer and effective alternative therapies[2,3].Natural polymers and nanomaterials have gained interest in cosmeceutical applications for their antimicrobial properties and biocompatibility. Chitosan, a naturally derived polysaccharide, exhibits broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria and fungi[4,5]. Additionally, it promotes wound healing, hydration, and serves as a film-forming agent, making it suitable for topical formulations[6,7]. rGO, due to its higher reduction potential and stability, effectively inhibits bacterial and fungal growth, making it a promising additive for topical acne treatment formulations[8,9]. However, conventional rGO synthesis methods often involve toxic chemicals, limiting their suitability for biomedical applications. The use of agro-waste for the green synthesis of rGO offers an eco-friendly, sustainable alternative aligned with the

principles of green chemistry[10,11,12].Peel-off gel masks have emerged as effective cosmetic delivery systems due to their ease of application, deep pore cleansing, and the ability to deliver active agents to the skin surface[13]. Given these advantages, the present study aims to develop and evaluate a peel-off gel mask incorporating rGO synthesized from rice husk agro-waste and chitosan, using Box-Behnken design for optimization.

METHODOLOGY

MATERIAL AND METHODS

MATERIALS

Rice husk was collected from local rice mill, potassium hydroxide and nitric acid were procured from Qualigens fine chem and SD fine chem limited, Mumbai, respectively. Lemon juice (extracted from lemons purchased from local market). Chitosan was obtained as a gift sample from Shilpa Medicare Pvt limited. Glycerol and lactic acid were purchased from Himedia and Merck specialties Pvt limited, Bengaluru, respectively. Nutrient broth and nutrient agar media were procured from Himedia, Bengaluru. Mueller-Hinton agar media was purchased from Sisco Research Laboratories Pvt limited, Mumbai.

METHODS

FORMULATION DEVELOPMENT

SYNTHESIS OF GRAPHENE OXIDE USING AGRO-WASTE:

In the present study, rice husk was selected as an agro-waste material for the eco-friendly synthesis of reduced graphene oxide (rGO) by the thermochemical process. Rice husk was collected from a local rice mill and washed with distilled water. It was dried in sunlight for a few days and finally dried in a vacuum oven at 50ºC for 12 h. The dried rice husk was crushed and ground well-using mortar pestle. About 3 grams of rice husk powder was weighed and transferred into a porcelain crucible and heated inside the muffle furnace (Heat control instruments and services) for 45 min at 450ºC. The solid residue obtained was called rice husk ash (RHA). After pyrolysis, the RHA was exfoliated using 2M nitric acid to remove impurities, followed by washing with distilled water until the pH became neutral and dried at 400ºC. The method mentioned above was slightly modified to produce porous GO sheets that enable more significant drug loading, including KOH activation of RHA. The RHA was mixed with KOH in the ratio of 1:2, followed by grinding for 15 minutes using mortar and pestle. The mixture of RHA and KOH was transferred into a porcelain crucible. The crucible was placed in the muffle furnace for 30 min at 400ºC, exfoliated using 2M nitric acid, washed with distilled water, and finally dried at 37 °C in a vacuum oven to get GO[14].

Experimental design for the synthesis of rGO

A two-level factorial design was chosen to get different experimental runs using Design expert software version12. The lower and higher levels of independent factors are given in the table. The independent variables and the dependent variables selected were the concentration of GO, the volume of lemon juice (LJ), reaction time (RT), and the absorption maxima (λ max) of rGO, respectively.

Table 1: Two-level factorial design for rGO

Std	Run	Factor 1 A: GO (mg/ml)	Factor 2 B: LJ (ml)	Factor 3 C: RT (h)	Response λ_{max}
6	1	5	5	48	269.0
8	2	5	20	48	270.80
7	3	1	20	48	269.20
1	4	1	5	6	278.0
4	5	5	20	6	211.20
2	6	5	5	6	212.80
5	7	1	5	48	270.0
3	8	1	20	6	281.0

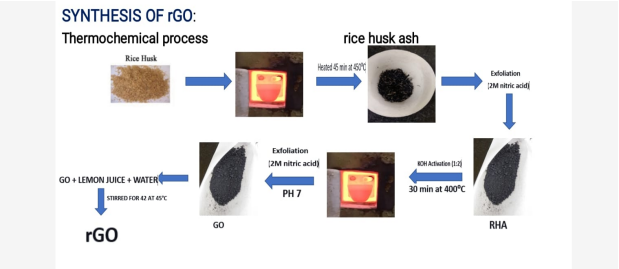


Figure-1: Synthesis of rGO

Method for synthesis of rGO

GO with a concentration ranging between 1 and 5 mg/ml was prepared in water, to which variable proportions of lemon juice was added at a temperature of 40ºC and stirred for varying periods using a magnetic stirrer (Remi) to get rGO which in turn was filtered and washed with water to remove impurities and dried at 45°C.

CHARACTERIZATION OF rGO

UV spectrophotometric analysis

10mg of rGO was accurately weighed and dispersed in 10ml of distilled water and sonicated for 10min to get a concentration of 1mg/ml (Stock Solution I). 2.5ml of SS I was diluted to 25ml using distilled water to get SS II with a concentration of 100µg/ml. An aliquot of 2.5ml of SS II was further diluted to 25 ml using distilled water in a volumetric flask to obtain a concentration of 10µg/ml. The resultant solution was scanned for wavelength of maximum absorption (λmax) between 300-400nm using Shimadzu UV-Visible spectrophotometer.

Statistical optimization

The wavelength of maximum absorption (λmax) was selected as a response variable. The response variable was statistically analyzed through analysis of variance (ANOVA) based on p-value and F value with the aid of design expert software version 12. Further, each factor and the response were subjected to multiple regression analysis to generate a polynomial equation. After analyzing the response, it was optimized employing numerical and graphical optimization technique to identify the optimum combination of factors.

Scanning Electron Microscopy

The shape and surface characteristics of the synthesized GO and rGO were studied using a scanning electron microscope (SEM). The samples for SEM were prepared by sprinkling the products on both-sided adhesive tape stuck to a stub. The coated substances were then randomly scanned, and photomicrographs were taken with an SEM[15].

Energy-dispersive X-ray analysis (EDAX) analysis

The elemental composition of GO and rGO was determined by EDAX analysis which indicates the percentage of each element present in the sample[16].

Particle size analysis

Particle size distribution and polydispersity index were determined by Zeta sizer version 7.12 (Malvern instruments Ltd.). The optimized rGO was reconstituted with 10 ml of Milli-Q water and diluted 10-fold with the same water using a vortex mixer. The measurements were made in triplicate and simultaneously standard deviation and standard error mean were calculated for each parameter determined.

Zeta potential analysis

The zeta potential of the optimized formulation was measured using Zetasizer Ver. 7.12analyzer, rGO was diluted in the ratio of 1:10 with Millipore water, vortexed for a minute, and subjected for analysis. Measurements were made in triplicate, standard deviation and standard error mean was calculated[17].

Fourier transform infrared spectroscopic (FTIR) analysis

FTIR analysis of synthesized rGO was carried out using Shimadzu FT-IR spectrophotometer. The potassium bromide (KBr) pellet method was employed for the study. The samples were thoroughly blended with dry potassium bromide crystals. The mixture was compressed to form a disc. The disc was placed in the sample cell and the spectra were recorded between 400-4000 cm^{-1}. The FTIR spectra of the pure drug and the optimized formulation were recorded and compared with each other[18,19].

DSC STUDIES

The thermogram of synthesized rGO was recorded using a differential scanning calorimeter (Perkin-Elmer-4000 series). Samples of rGO were placed on aluminum pans and thematically sealed. An empty pan sealed in the same way as wwfor the sample was used as a reference. The thermal behavior of the samples was investigated under nitrogen gas at a scanning rate of 10°C per minute, over a temperature range of 25-450°C[20].

XRD analysis

XRD analysis of rGO was performed using Goniometer (PW3050/60 theta/theta gonio with 40 KV/30mA - X-Ray generator settings, 2θ/θ scanning mode with fixed monochromator). Data were obtained for the 2θ range of 10 to 79.98 with a step of 0.03 degrees[18,19].

FORMULATION OF PEEL-OFF GEL MASK

FORMULATION DESIGN:

Different formulations of peel off mask was prepared by applying Box Behnken design using design of experiments software, Design expert version 12. The independent variables selected were, polymer concentration (chitosan), the concentration of glycerol, and the volume of ethanol. The response variables selected were drying time, dispersion of gel, thickness of film and folding endurance. The levels and factors are given in the design table. The lower and higher levels of independent factors were selected based on the reported literature and initial screening experimental conducted.

Table no. 2: Box-Behnken response surface design for preparation of peel off gel mask with response values

Std	Run	Chitosan (g)	Glycerol %w/w	Ethanol (ml)
1	3	0.5	10	25
3	12	0.5	30	25
8	2	1.5	20	40
12	1	1	30	40
14	6	1	20	25
15	4	1	20	25
13	7	1	20	25
11	16	1	10	40
16	10	1	20	25
6	11	1.5	20	10
10	5	1	30	10
9	15	1	10	10
5	8	0.5	20	10
2	9	1.5	10	25
7	13	0.5	20	40
4	14	1.5	30	25

Method of preparation:

Formulation of chitosan films

Varying volumes of ethanol ranging between 10-30ml as specified in the table were mixed with 70 – 90 ml water, to which 1 ml of lactic acid, specified quantities of chitosan, and glycerol (table 2) were added with continuous magnetic stirring to get a homogenous dispersion. Rosewater was added as perfume. 10 ml of the dispersion was poured into Petri plates and dried in the vacuum oven until complete removal of moisture and films were peelable. The drying time for each formulation was noted in (table no.2).

CHARACTERIZATION OF PEEL-OFF GEL

Dispersion of gel: The gel dispersion of 0.2 ml was dropped on the glass plate and pressed with another glass plate by placing a weight of 500g. After 5 min, the glass plate was removed, and the diameter of the dispersion of gel was measured.

Drying time: The 10 ml of gel dispersion was poured into the Petri plates and dried using a vacuum oven (make). The drying time was noted for each of the film formulations.

Thickness of film: The thickness of the film was measured using a screw gauge. Average measurements at three points for each film formulation were done to determine the overall thickness of the formulation[19].

Folding endurance: a uniform cut out of the film was taken and folded back and forth until the film got torn. The count of film folding was noted down at which the film broke[20].

Statistical optimization

The response variables selected for optimization were drying time, dispersion of gel, the thickness of the film, and folding endurance. The response variables were statistically analyzed using ANOVA based on p-value and F-value using design expert software, version 12. Each parameter and the responses were subjected to multiple regression analysis to generate polynomial equations. After analysing, the response variables were subjected to multiple response optimization utilizing numerical and graphical optimization to identify the optimum combination of factors that would simultaneously optimize multiple responses[17].

Table 3: Optimized formulation

Ingredients	Quantity
Chitosan (g)	1.379
Glycerol (%w/w)	13
Ethanol (ml)	23

Formulation of rGO and chitosan films

rGO and chitosan dispersion prepared using respective optimized formulas were used for preparing rGO loaded chitosan films. 1g of rGO was mixed with 100 ml of chitosan dispersion prepared by the above-mentioned method.

Determination of pH

The pH of the rGO loaded optimized gel dispersion was determined by using a pH meter.

Antimicrobial activity:

All the apparatus and the media used in the study were sterilized by autoclaving at temperature and pressure of 121ºC and 15lb/in^2, respectively, for 15 min.

Test organisms

The test organisms used in the study were Escherichia coli ATCC8739, *Staphylococcus aureus* ATCC6538, *and Candida albicans* ATCC1023.

Culture media

The nutrient broth was used for preparing the stock microbial suspension, and nutrient agar was used to prepare agar slants and agar plates during the sub-culturing of microorganisms. Muller Hinton agar media was used for antimicrobial effectiveness testing.

Preparation of agar plates

Accurately weighed 1.95 g of Muller Hinton agar media was taken in 500 ml of distilled water in a conical flask and allowed to dissolve completely with the aid of heat. Stoppered the conical flask with cotton plug and sterilized by autoclaving. After sterilization, 20 ml of molten agar was poured into Petri plates of 95 mm diameter in the aseptic area, allowed to solidify. As soon as the agar solidified, vapours at the underside of the lid were removed with sterile cotton, and the lid was closed.

Preparation of the inoculum

Three to five well-isolated colonies of the same morphological type from the agar plate of Staphylococcus aureus, Escherichia Coli, and Candida albicans were selected. Each colony was touched with a sterile inoculating loop and transferred separately into a 5 ml nutrient broth medium in culture tubes. The broth culture tubes were incubated at 35±2ºC until adequate turbidity was achieved. The turbidity of the microbial suspension was adjusted by serial dilution method using sterile nutrient broth. 1ml of microbial suspension from fresh broth culture was transferred to 9 ml sterile water and continued subsequent dilution until the turbidity of the suspension matched with 0.5 McFarland's standard reagent.

Cup plate or agar diffusion method

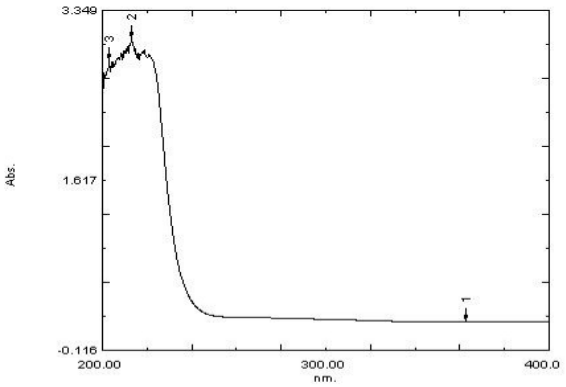
In this method, optimally, within 15 min after adjusting the turbidity of the inoculum suspension, 200µl of the inoculum suspension was spread on the solidified agar surface using a glass spreader. The lids were closed and labeled before being the wells were made. The cavities of 15mm were made on the agar surface using a sterile cork borer. 200 µl of chitosan gel dispersion (CD), reduced graphene oxide loaded chitosan gel dispersion (rGCD) were instilled into the respective cavities using a sterile micropipette. Similarly, dried chitosan film (CF) and reduced graphene oxide loaded chitosan films (rGCF) of 1cm×1cm were cut with sterile scissors and placed on the inoculated agar surface. One of the inoculated agar plates was kept as a positive control, and another agar plate without the inoculum was treated as a negative control. The Petri plates treated with gel dispersion and films were left in the aseptic area for one hour to diffuse the formulation across the solidified agar. Then, all the plates were placed in the incubator in the inverted position with a temperature of 35±2ºC for 48h. After 48h of incubation, the plates were observed for the zone of inhibition, measured using a scale, and recorded.

Results and Discussion

Analytical method

Characterization of GO and rGO

UV Spectroscopic analysis of GO and rGO:



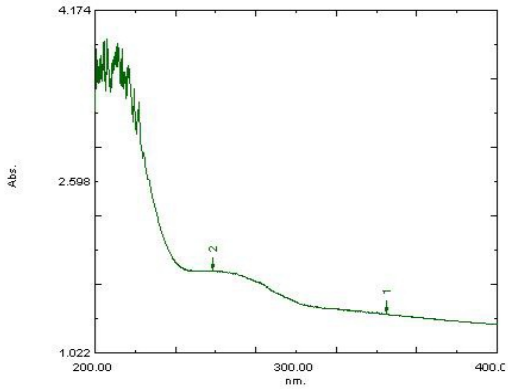


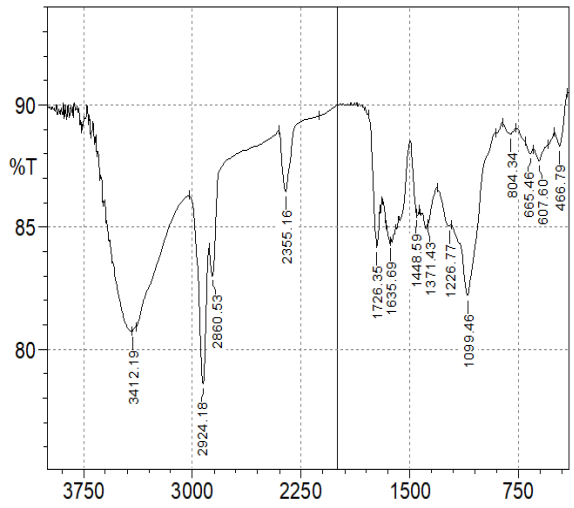
Figure-2: UV Spectrum of GOFigure-3: UV Spectrum of rGO

Λmax for GO = 212 nm

Λmax for rGO = 258 nm

Graphene oxide (GO) was successfully synthesized from rice husk using a green thermochemical method, followed by its reduction to rGO using lemon juice as a natural reducing agent. UV-Vis spectroscopic analysis of GO showed a λmax at 212 nm, confirming its structure, while the reduced form showed red-shifted λmax values ranging from 258–281 nm, indicating successful deoxygenation and restoration of sp^2 carbon domains.

FTIR analysis of GO and rGO:



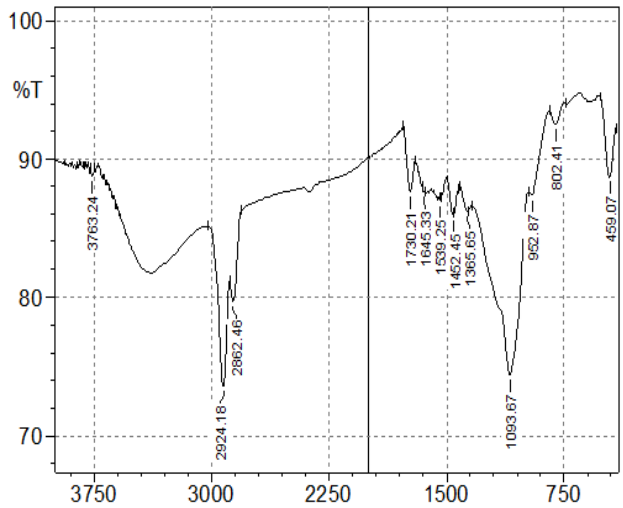
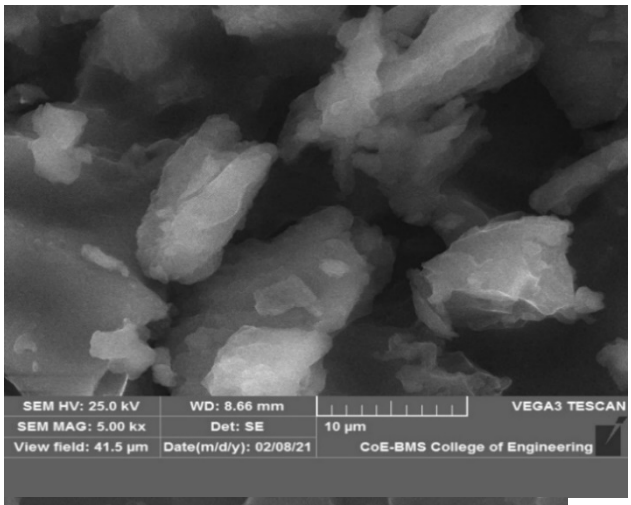


Figure-4:FT-IR Spectra of GOFigure-5:FT-IR Spectra of rGO

The FT-IR spectrum of GO showed the characteristic absorption bands of hydroxyl, carboxyl, and epoxide groups. The spectrum confirms the oxidation of graphite to graphene oxide. Presence of oxygen-containing groups like –OH, C=O, COOH, C–O, and epoxy groups show successful functionalization. The broad –OH band at ~3412 cm^{-1} and carbonyl peak at ~1728 cm^{-1} are classic indicators of GO. Residual C=C peaks (around 1626 cm^{-1}) show that some graphitic structure remains.The FTIR of rGO confirms successful reduction of GO. Oxygen-containing groups (–OH, C=O, C–O) are partially removed, as seen by the decreased intensity of these peaks. C=C stretching becomes stronger, meaning the sp^2 graphitic network is being restored.

Scanning Electron Microscopic (SEM) analysis of GO:



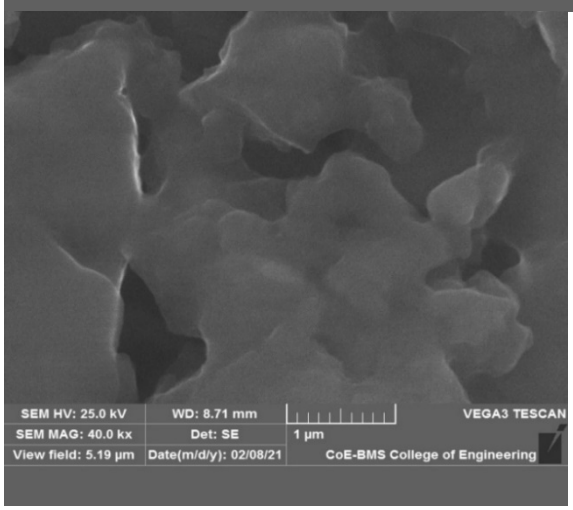
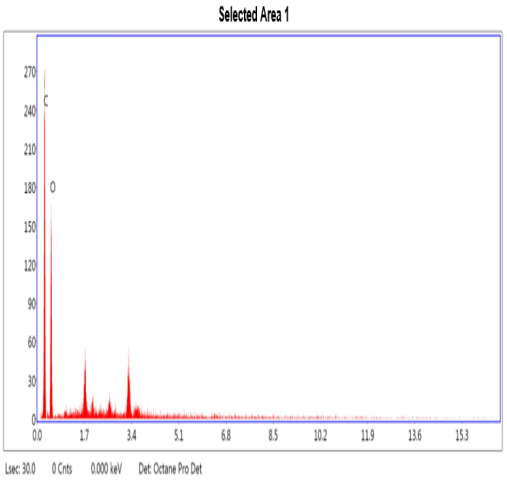


Figure-6: SEM Images of GO

EDAX (Energy- Dispersive X- ray Spectroscopy)Of GO and rGO



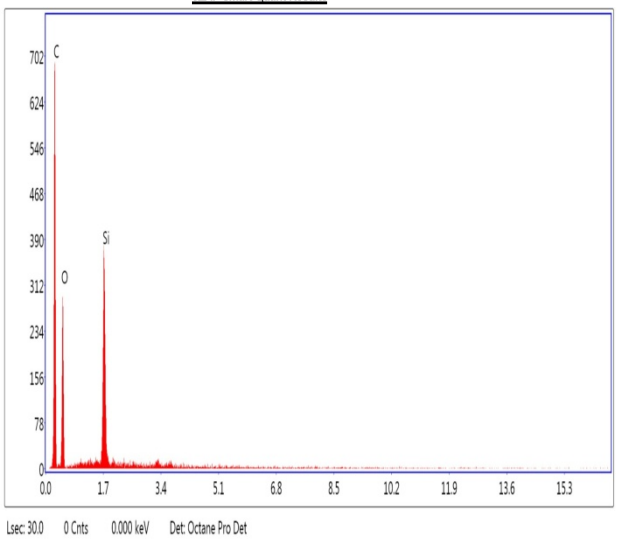


Figure-7: Selected area for EDAX of GO
Figure-8:Selected area for EDAX of rGO

Table 4: Data showing elemental composition of rGO

Element	Weight %	Atomic %
C	53.38	60.40
O	46.62	39.60

Element	Weight %	Atomic %
C	60.75	68.55
O	34.34	29.08

EDAX analysis showed an increase in carbon content post-reduction, confirming efficient removal of oxygen-containing groups. SEM images revealed a transformation from crumpled, aggregated GO sheets to smooth, stacked multilayered rGO sheets.

Particle size Analysis of rGO:

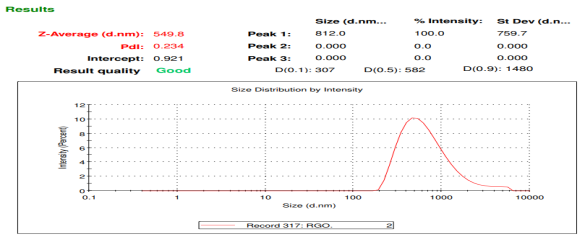


Figure-9: Particle size analysis report of rG

Zeta potential analysis of rGO:

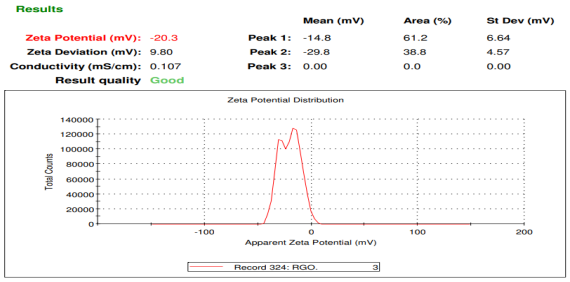


Figure-10. Zeta potential analysis report of rGO

XRD Analysis

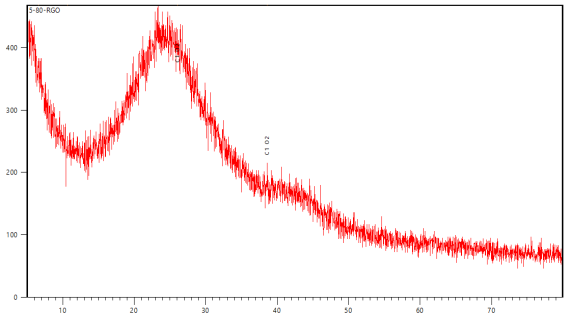


Figure-11: XRD pattern of rGO

XRD analysis of rGO revealed diffraction peaks at 2θ values of 26.03° with high intensity of 138.88 and 10.62° with intensity 6.18

The average particle size of rGO was found to be 556.2 nm with a PDI of 0.238, indicating uniform dispersion. The zeta potential was −19.86 mV, which suggests moderate colloidal stability. XRD analysis further confirmed the structural reduction, with the characteristic GO peak (2θ ~10°) shifting towards ~26°, typical for rGO.

Differential scanning calorimetry (DSC):

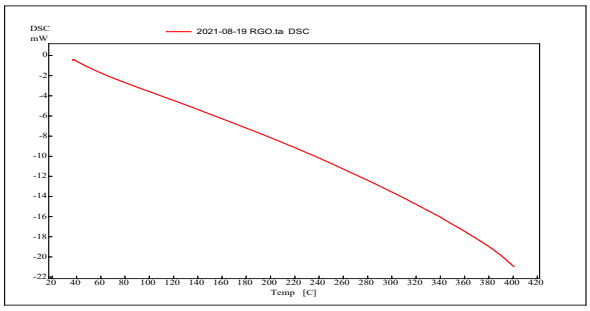


Figure-12: DSC thermogram of rGO

Table 5:EVALUATION OF PEEL OFF GEL MASK

Formulation code	Drying time (h)	Dispersion of gel (cm)	Thickness of film (mm)	Folding endurance (number of foldings)
POGMCH 3	0	4.4	0	0
POGMCH 12	72	4.53	0.12	19
POGMCH 2	2.5	3.9	0.116	47
POGMCH 1	2.5	4	0.116	53
POGMCH 6	4.5	4.1	0.116	54
POGMCH 4	4	4.1	0.123	58
POGMCH 7	3.5	4.1	0.117	76
POGMCH 16	3.5	4.13	0.136	90
POGMCH 10	3	4	0.126	93
POGMCH11	4	4.43	0.143	104
POGMCH 5	72	3.73	0.02	119
POGMCH 15	3.5	4	0.136	121
POGMCH 8	3	4.2	0.116	122
POGMCH 9	4	4.33	0.133	124
POGMCH 13	2.5	4.53	0.15	124
POGMCH 14	2.5	4.46	0.046	153

Response 1: Drying time

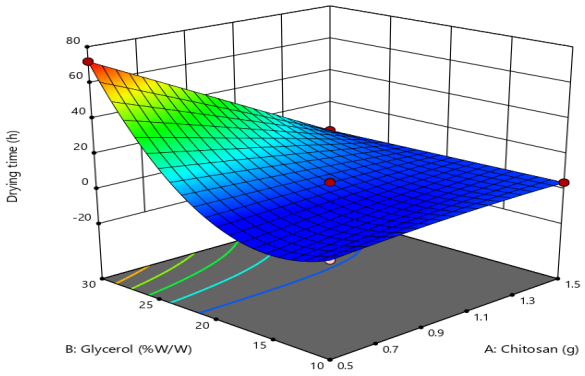


Figure-13: response graph showing effect of glycerol and chitosan on drying time

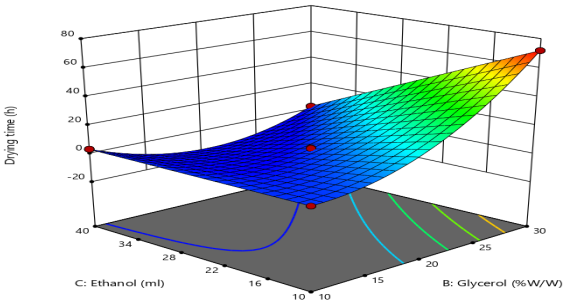


Figure-14: response graph showing effect of ethanol and glycerol on drying time

Time required for drying the film was found to increase with an increase in the concentration of chitosan and glycerol and decrease with an increased concentration of ethanol.

Response 2: Dispersion of gel

Dispersion of gel indicates the spreadability of the dispersion on application. Higher concentrations of polymer and glycerol have shown decreased spreadability due to the dispersions increased viscosity. An increase in alcohol concentration has increased the spreading property of dispersion because of reduced viscosity.

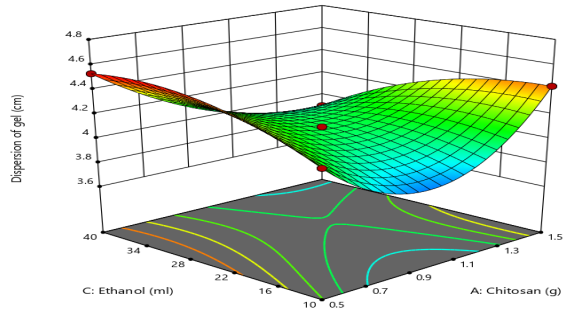


Figure-15: response graph showing effect of ethanol and chitosan on dispersion of gel

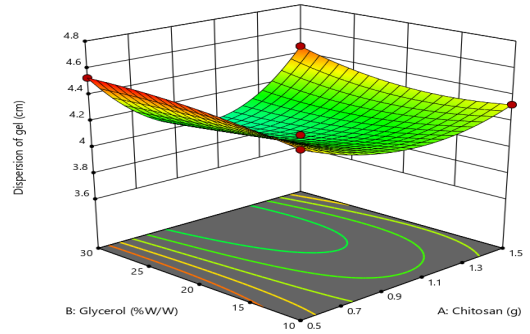


Figure-16: response graph showing effect of glycerol and chitosan on dispersion of gel

Response 3: Thickness of film

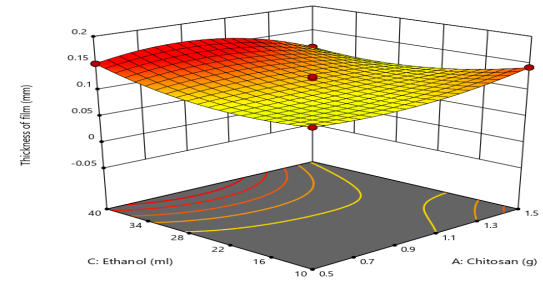


Figure-17: response graph showing effect of ethanol and chitosan on thickness of film

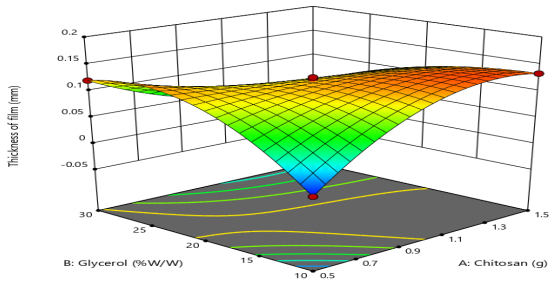


Figure-18: response graph showing effect of glycerol and chitosan on thickness of film

Response 4: Folding endurance:

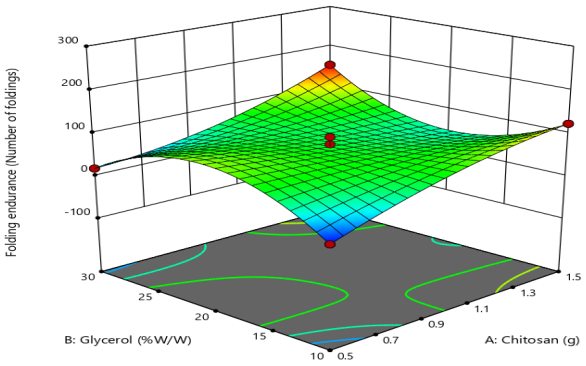


Figure-19: response graph showing effect of glycerol and chitosan on folding endurance

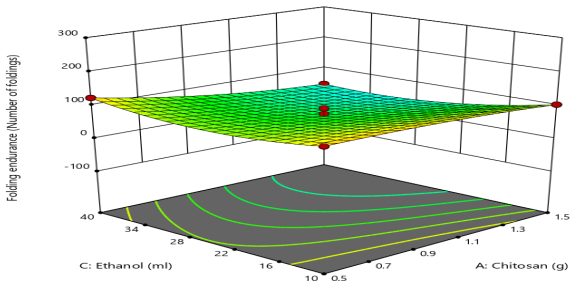


Figure-20: response graph showing effect of ethanol and chitosan on folding endurance

Drying Time

The drying time ranged from 2.5 to 4.5 hours across formulations. ANOVA revealed that glycerol and ethanol had a statistically significant effect on drying time ($p < 0.0001$). Increased ethanol concentration decreased drying time due to its high volatility, while increased glycerol and chitosan content prolonged it due to enhanced viscosity and polymer density.

Gel Dispersion

Dispersion diameter ranged from 3.73 cm to 4.53 cm. Ethanol positively influenced dispersion by reducing viscosity ($p = 0.0229$), while higher concentrations of chitosan and glycerol reduced spreadability. The optimum formulation exhibited a desirable spreadability of 4.35 cm.

Film Thickness and Folding Endurance

The film thickness varied between 0.02–0.15 mm. Glycerol and ethanol significantly influenced this parameter ($p < 0.005$). Folding endurance values ranged from 19 to 153, where higher chitosan concentration led to more flexible films, attributed to better polymer cross-linking and cohesive strength.

Table06: Predicted and actual values of selected response variable:

Response Variables	Predicted	Actual
Drying time (h)	3	3
Dispersion of gel (cm)	4.25	4.35
Thickness (mm)	0.119	0.115
Folding endurance (number of foldings)	101	105

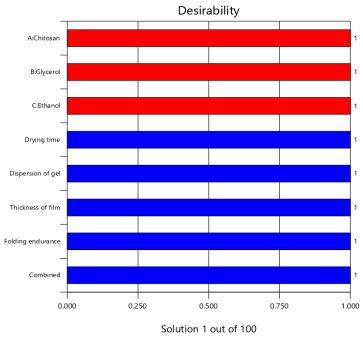


Figure-21: Bar graph of desirability (numerical optimization)

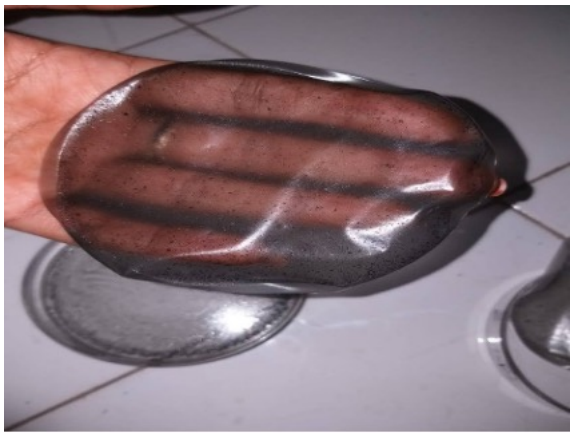


Figure-22: Photographs optimized formulation rGO loaded peel off gel mask

Evaluation of Optimized rGO-Chitosan Gel Mask

The optimized formulation (1.379 g chitosan, 13% glycerol, 23 mL ethanol) demonstrated thefollowing responses: 3 h drying time, 4.35 cm dispersion, 0.115 mm thickness, and 105 foldings. These matched closely with predicted values, validating the Box-Behnken model.SEM analysis showed that the rGO-loaded chitosan films had smoother and denser microstructures than unloaded films, indicating improved film-forming properties and surface uniformity.

Results of antibacterial and antifungal activity

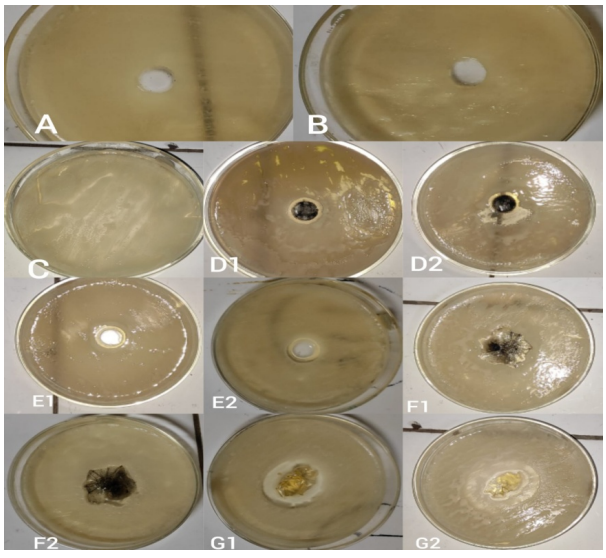


Figure-23: Images of zone of inhibition (*Escherichia coli ATCC8739)*

A- reduced graphene oxide 200µ g
B- reduced graphene oxide 500µg
C- positive control
D1 & D2- graphene dispersion
E1 & E2- chitosan dispersion

F1 & D2- graphene films
G1 & G2- chitosan films

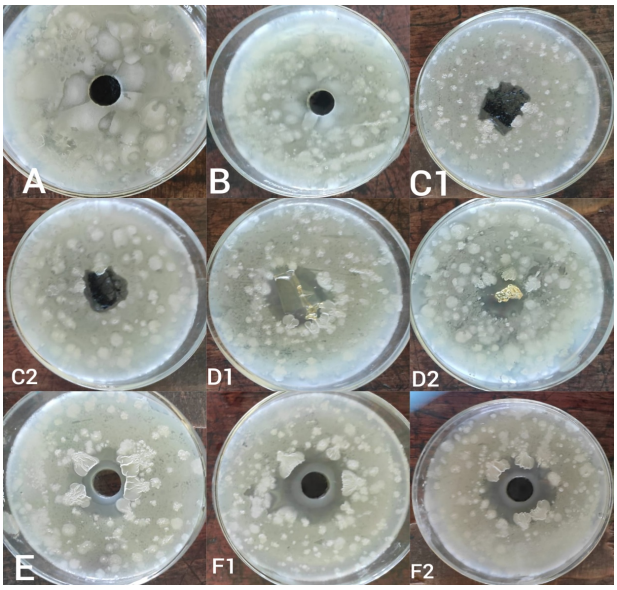


Figure-24: Images of zone of inhibition (*Staphylococcus aureus ATCC6538)*
A- Reduced graphene oxide 10000µg
B- Reduced Graphene oxide 1000µ g
C1 & C2 - Graphene Film
D1 & D2 - Chitosan Film
E - Chitosan dispersion
F1 & F2 - Graphene dispersion

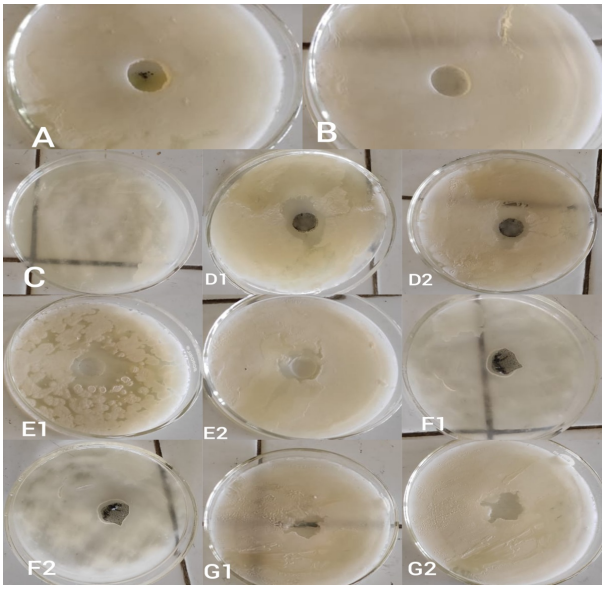


Figure-25: Images of zone of inhibition (*Candida albicans ATCC1023)*
A- Reduced graphene oxide 200µg
B- Reduced graphene oxide 500µg
C- Positive control
D1 & D2 - Graphene dispersion
E1 & E2 - Chitosan dispersion
F1 & F2 - Graphene films
G1 & G2- Chitosan films

Antimicrobial Activity

The antimicrobial efficacy was assessed using the agar diffusion method against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. The rGO-loaded chitosan film (rGCF) showedenhanced activity with inhibition zones of 4.05 ± 0.071 cm (S. aureus), 4.00 ± 0.282 cm (*E. coli*), and 3.65 ± 0.071 cm (*C. albicans*), compared to the chitosan film alone (CF) and gel dispersions(CDandGCD).These findings align with literature reporting the synergistic antimicrobial properties of chitosan and graphene derivatives due to disruption of microbial membranes and oxidative stress mechanisms. rGO's sharp edges and redox-active sites, along with chitosan's polycationic nature, enhance microbial interaction and disruption, particularly against Gram-positive organisms.

Conclusion

The present study successfully demonstrated the synthesis of reduced graphene oxide (rGO) from rice husk via an eco-friendly thermochemical process and its effective incorporation into a chitosan-based peel-off gel mask. The optimization of both rGO synthesis and peel-off gelformulation using factorial and Box-Behnken designs provided robust predictive models withhigh desirability scores. Characterization studies confirmed the structural integrity, particle size uniformity, and stability of the synthesized rGO.The optimized rGO-chitosan peel-off gel mask exhibited favorable physicochemical characteristics, including ideal drying time, spreadability, film thickness, and folding endurance. More importantly, the formulation showed significant antibacterial and antifungal activities against *Staphylococcus aureus, Escherichia coli,* and *Candida albicans,* underscoring its potential as an effective cosmeceutical product for acne and other skin-related infections.This research validates the integration of natural polymers and nanomaterials in topical delivery systems and offers a sustainable approach to graphene synthesis using agricultural waste. The developed formulation holds promise for further clinical evaluation and potential commercial application.

Conflict of Interest

The authors declare no conflict of interest related to this research work. The study was conducted independently without any financial or commercial influences that could potentially bias the results or interpretations.

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