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Quality by design approach for the synthesis of graphene oxide nanosheets using full factorial design with enhanced delivery of Gefitinib nanocrystals

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Abstract

Designing drug delivery carriers is the most focused work for a material scientist. The formulator can screen the material starting from its properties to the performance of the material. The quality by design approach has simplified the path of selection of the right parameter for analyzing the process. The present investigation elaborates the use of a full factorial design model for understanding the interaction of oxidizing agents on the conversion of graphite to graphene oxide (GO). The most frequently assessable laboratory method is chemical oxidation, which is used for understanding optimum oxidation potential and nanosheet formation. The method utilizes 2 level assessments for screening reactant concentration of sulphuric acid and potassium permanganate on preprocessed graphite. In present investigation, one categorical factor is used to understand the effect of precursor size on the final product. The statistical model provides optimum oxidation conditions, using particle size, polydispersity index (PDI), and ID/IG ratio with a 95% confidence interval (p-value less than 0.05). The optimized synthesis procedure provides the least particle size of GO nanosheet of about 220.7 nm with PDI 0.289 and ID/IG ratio of 0.98. Furthermore, pulse mode ultrasonication converts Gefitinib (GF) into nanocrystals and is deposited within intricates of GO nanosheets (nGOGF). The GO and nGOGF were preliminarily characterized using optical and vibrational spectroscopy. The hydrodynamic diameter was found to be slightly increased to 237.5 nm with decreasing surface charge (−33.64 mV) after fabrication. The x-ray Photoelectron Spectroscopy (XPS) study reveals successful grafting of oxygen-containing functional groups on GO nanosheets with peak positions observed at 284–288 eV. The Transmission electron microscopic (TEM) observation supports the wrinkled structure of GO nanosheets synthesis, along with encapsulation of GF nanocrystals. The nGOGF retard the release of GF for a prolonged period of time and the rate of dissolution was increased by fold compared to pure GF.

1. Introduction

Designing a drug delivery carrier is a very critical step and requires technological assessment along with appropriate facilitation. Many scientists uses laboratory based approaches to selectively synthesize the nano-based carrier for the delivery of the therapeutic agent. Synthesis of carbon backbone-based material has been explored tremendously for delivery and diagnosis during the last decade [1]. Compared to polymeric or metal nanoparticle synthesis, carbon backbone-based synthesis approaches are elucidative and require a few steps. The path of synthesis and verification is very expository and need to screen every step crucially for designing the final
output. A researcher would specifically select laboratory chemical oxidation method or mechanical exfoliation as one of the protocol for initial screening [2]. At least 2% of researchers fail to get positive results due to critical process parameters and unmatched needs to satisfy quality attributes. Many formulators and academic scientists prefer to use chemical oxidation processes and satisfy the unmatched needs of the delivery system. Sources of the precursor may be different and it is very critical to decide at the initial stages of screening, which could be more productive. In addition to this, pertinent concentration ratios of reactants may not be easily discoverable.

Oxidized graphite has hybridized (sp²-sp³) structure available for interaction with adjacent molecules. The promoted oxygen-containing functional group helps to implicate hydrophilicity and is able to be dispersed in many solvents including water [3]. The expanded interlayer spacing or conversion of stacked graphene sheets to single sheets would be channelized using optimum synthesis protocol [4].

Forthcoming development in the synthesis of nanocarrier is the use of the quality by design (QbD) approach. The combination of factors and response can be statistically screened, which could assist in understanding the interaction and multiple variables at a single time point. The QbD provides an excellent opportunity for the selection of an appropriate concentration of variable from a limited number of experiments [5, 6].

Very less number of literature refers to the application of QbD during chemical synthesis process. Many scientists explored QbD for preparation, synthesis of nanocomposites [7], mixtures [8], materials complexes [9], hybrid structure [10], etc using graphene as substrate. de Araujo C MB et al investigated the systematic approach for the synthesis of graphene oxide (GO) using fractional factorial design. The process parameters such as effect of reaction time, volume of sulphuric acid and ultrasonication were investigated using statistical approach. The output response was selected as percentage removal of methylene blue dye. Total 4 number of runs estimated from 2³ fractional factorial design matrix. The volume of sulphuric acid was reduced to 23 ml with additional 2 ml water used during synthesis. Optimization points out the use of lower volume of sulphuric acid, no use of ultrasonication and reaction time reduced to 3 h. The percentage of dye removal was over 99% enhances sorption capacity due to increase in the surface area [11].

The GO synthesis process was analyzed using TOPSIS Taguchi design helps to screen the relationship between variables and properties. Over the multiples of oxidation responses, graphene defect, surface roughness, crystallite size were selected. Centrifugation causes highest variation during synthesis of GO and optimally screen using statistical support. Total 7 factors were screened for GO and rGO synthesis respectively. The centrifugation, concentration of potassium permanganate causes major variations in the synthesis of GO. While for rGO synthesis, the amount of GO, ultrasonication time, type of heating have shown high variability during the reduction process [12].

Chemical vapor deposition is the most advanced technique use for the synthesis of GO. Design of experiment was used to quantitatively analyzed the process parameters to obtain good quality GO. The source of carbon (1–3 benzene ring in carbon precursor), temperature (800 °C–1000 °C) and mass flow rate (14–57 mg min⁻¹) were considered as independent variable. The crystallinity and number of layers of graphene were set as responses obtained from micro-Raman analysis. The 3 level Box–Behnken design was selected to optimize the synthesis protocol. The copper foil act as catalyst during synthesis of GO with additional controlling factor required such as atmospheric pressure (1 atmosphere) The process finds generation of single layer graphene in the presence of homologous carbon precursor [13].

The GO is one of the best suitable carriers selected for the delivery of the drug. Due to embedded oxygen-containing functional groups, high payload capacity, the honeycomb-like structure, high mechanical stiffness, multiple payloads, etc makes graphene a more attractive material for biomedical applications [14]. The multiple layer arrangement, high entrapment of drug, haemocompatibility, biocompatibility, non-irritant, easy transportation across the cell membrane, protection from degradation, etc provides an attractive candidate for the selection of drug delivery carrier [15]. The graphene interacts with therapeutic molecules in multiple ways either via π-π* stacking interaction, Van der Waals interaction, stearic bonding, covalent bonding, ionic bonds, etc [16].

Chemical oxidation of graphite to GO is a very critical step. The minute change in the concentration of oxidizing agent may change the oxidation state of GO [17]. One more parametric determination analyzed during the synthesis process is the particle size of the precursor. Before processing for the reaction, one must verify the particle size of the precursor and decide the concentration of the oxidizing agent. Minute variation in the concentration of oxidizing agents may lead to the direct conversion to reduced graphene oxide (rGO) [18]. For a pharmaceutical application or in many specialized applications, the oxygen surface functionality of graphene needs to be preserved at an optimum level. The interactive oxygen surfaces are useful for conjugation of biomolecules, grafting of drugs, prodrugs, antibodies, enzymes, amino acids, etc. The GO is also helpful in biosensing applications with embedded oxygen functionality [1]. The conduction and transport properties of GO may vary in comparison to rGO [19]. Sometimes drug loading capacity decreased after the formation of rGO- due to a decrease in aromatic interaction [20].
Table 1. Design table and experimental parameters in $2^3$ full factorial design.

| Run sequence (Batch) | Independent variables | Pot permanganate (Numerical) |
|----------------------|-----------------------|-----------------------------|
|                      | Graphite size (Categorical) | Sulphuric acid (Numerical) |
| 1                    | 10 Micron             | $-1$                        |
| 2                    | 8 Micron              | $-1$                        |
| 3                    | 8 Micron              | $1$                         |
| 4                    | 10 Micron             | $1$                         |
| 5                    | 10 Micron             | $-1$                        |
| 6                    | 8 Micron              | $-1$                        |
| 7                    | 10 Micron             | $1$                         |
| 8                    | 8 Micron              | $1$                         |

The present context of investigation deals with the screening of most appropriate concentration of reactant required for oxidation of graphite to GO. The 2 level full factorial design is used for understanding the oxidation potential of the reactant. The statistical summary investigates the optimum level of reactant which converts graphite to GO nanosheets. The particle size, polydispersity index (PDI), and defect level ($I_D/I_G$ ratio) were considered as a response. The response optimizer predicts the direct formation of GO nanosheets within the given design space. The nanosheets formed were analyzed for surface morphology and internal structure. The statistically designed and verified GO nanosheets were used for the delivery of Gefitinib (GF) nanocrystals. The attempts provide enhancement in release characteristics of GF nanocrystals.

2. Materials and methods

Graphite was provided as a generous gift sample by Asbury Carbons, New Jersey, USA. The supplier has provided technical datasheets along with variable particle size samples (8, 10 microns). Gefitinib (GF) was received as a gift sample from BioXpert Innovations Pvt. Ltd India. The Potassium permanganate, Sulphuric acid, Sodium nitrates were purchased from Sigma Aldrich, India. Hydrogen peroxide, Hydrochloric acid, etc were procured from Merck specialties Ltd Mumbai, India. The chemicals and solvents used during the synthesis and preparation of materials were used without further purification.

2.1. Optimization protocol for the synthesis of graphene oxide (GO) nanosheets

The optimized synthesis protocol was designed by screening variables using a statistical approach. The different batches were predicted using 2 levels of full factorial design and represented in table 1 (coded values). The optimized synthesis protocol was started with microwave irradiation of 1:0.2 ratios (w/w %) of graphite precursor and sodium nitrate. Using conventional microwave, the mixture was irradiated at 250 W for 10 s per cycles for total of 120 s [21]. The after irradiation the mixture was cooled down to room temperature and 75 ml of concentrated Sulphuric acid was added. Stirred the reaction mixture for 10 min to make homogenous dispersion and transfer into an ice bath at a controlled temperature (below 10 °C). Start addition of 9 g Potassium permanganate at controlled temperature environment. Continue the reaction for 3 h or until viscous slurry forms. Start slower addition of 70 ml distilled water (DW) while maintaining the temperature below 10 °C. After complete addition, provide heat up to 60 °C for 60 min and stirred to regain the temperature to normal. Add remaining 150 ml water and add slowly hydrogen peroxide from the sidewall of the Erlenmeyer flask. A golden yellowish dispersion form confirms the optimum oxidation of graphite. Continue the stirring for a further 2 h and centrifuge at 5000 rpm for 20 min. The supernatant was discarded and the solid was redispersed into 10% HCl solution and centrifuged at 10,000 rpm for 15 min. Finally, wash with DW till neutralization using centrifugation process at 20,000 rpm for 20 min. The neutral dispersion in DW was probe sonicated for 40 min. The dispersion was transferred to a hot air oven and dried at 40 °C. The dried flakes were collected and used for further analysis. The initial responses were recorded according to predicted batches provided by Minitab statistical analyzer (version 19).

2.2. Fabrication of Gefitinib nanocrystals loaded graphene oxide nanosheets (nGOGF)

A passive drug loading approach was used for the loading of Gefitinib (GF) on GO nanosheets. The synthesized GO nanosheets (10 mg ml$^{-1}$) were dispersed into DW. The dispersion was ultrasonicated for 20 min, promotes exfoliation of nanosheets. By using aromatic $\pi-\pi^*$ stacking interaction GF interacts with GO nanosheets and
exfoliated sheets entrap GF nanocrystals in presence of pulse mode ultrasonication. Ethanolic solution of GF (2 mg ml$^{-1}$) was added dropwise using an insulin syringe into previously exfoliated GO nanosheets dispersion. In the presence of probe sonication, drop-wise slower addition of GF converted to nanocrystals because of high-intensity cavitation. After complete addition, nGOGF was centrifuged at 20,000 rpm for 30 min and the supernatant was analyzed using a UV–vis spectrophotometer (for percent encapsulation). The nGOGF dispersion was washed with DW in triplicate and centrifuged at 20,000 rpm for 30 min. The solid collected after centrifugation was dried at 40 °C in the oven.

2.3. Characterization of synthesized GO and fabricated nGOGF

Preliminary characterization of the oxidation process was verified using Ultra Violet Visible spectroscopy (UV–vis) and Fourier Transform infrared spectroscopy (FTIR). The synthesis of GO nanosheets was a very critical process and needs extensive screening and verification at every stage of the oxidation reaction. The process of chemical oxidation was preliminarily identified using UV–vis Spectrophotometer (Shimadzu, 1800, Japan) by dispersing it into DW. The absorbance was recorded at every stage of reaction utilizing equivalent diluent volume for comparison. The passive loaded GF was also verified by determining % encapsulation efficiency and drug content by measuring UV–vis spectrophotometric absorbance. The FTIR analysis was performed using diffuse reflectance spectra (8400 s, Shimadzu, Japan) by mixing samples with predried KBr (1:100) respectively. Infrared radiation was directly incident on the sample and vibrational frequencies were analyzed within the range of 400–4000 cm$^{-1}$. The particle size, zeta potential, and PDI of synthesized batches of GO and nGOGF were understood using a particle size analyzer (NanoPlus 3, Particulate system, Micromeritics, USA). The x-ray diffraction analysis of samples was carried out using a D8 Advanced XRD analyzer (Bruker, Germany). The x-rays were incident on the samples at wavelength 0.154 nm (Cu-Kα). The fast counting detector fixed using silicon strip technology counts 2θ angles (Bruker Lynx Eye Detector). The carbon allotropes were analyzed using Raman spectroscopy. The Raman spectra identify the phase inversion, oxidation, interlayer spacing, etc of synthesized GO, GF and nGOGF. The analysis was performed on Horiba Jobin–Yvon, Hr 800 monochromator, USA with a scanning range from 500–3200 cm$^{-1}$. Surface morphological evaluation and elemental analysis of samples were performed using a field emission scanning electron microscope (S–4800, Hitachi, Japan, and Jeol SEM, Germany). The internal structure of synthesized nanosheets and nanocrystalline GF was analyzed using a High-Resolution Transmission electron microscope (HRTEM—Philips CM200). The x-ray Photoelectron Spectroscopy (XPS) analysis of synthesized GO was performed using Thermo fisher Instrumental System (ESCALAB 250i).

2.4. In-vitro drug release

The dialysis bag method was used for understanding the drug release characteristics of nanocrystalline GF from the intricate of GO [22]. The 10 mg equivalent of nGOGF was dispersed in 0.5% sodium carboxymethyl cellulose (NaCMC) dispersion. Previously dialysis bag was soaked in phosphate buffer pH 7.4 for 24 h and one side of the dialysis tube was closed using the clamp. The nGOGF dispersion was transferred to a dialysis tube and other end packed using another clamp. Dialysis bag was added into 250 ml phosphate buffer containing beaker, stirred at 100 rpm at controlled temperature 35 ± 0.5 °C. Pure GF was poorly soluble in phosphate buffer pH 7.4, for enhancing identification of drug, 0.2% of Sodium Lauryl Sulfate (SLS) was added as surfactant. During preparation, 10 mg of GF was dispersed into 0.5% NaCMC and transferred to another dialysis bag soaked previously for 24 h. At a predetermined interval of time, 3 ml of sample was withdrawn and maintained sink condition by addition of fresh phosphate buffer pH 7.4. The sample was diluted 10 times with Phosphate buffer pH 7.4 before analysis and absorbance was recorded. The plot of cumulative drug release versus time was used to determine the comparative release pattern of pure GF and nGOGF.

2.5. Statistical assessment for optimization of GO

The critical process attributes and critical quality attributes were screens from initial trial and error batches. Statistical analysis was done in a step-by-step manner using 2$^3$ full factorial designs. Estimation of interaction between selected factors and response, coefficient, formula, final prediction factors were investigated using statistical software Minitab (version 19). Chemical oxidation of graphite was a very critical and time-consuming process. Each stage needs to be handled very carefully and perform specific tactics while performing the reaction. Various categorical limitations (Covariates) were optimized and fixed during process optimization like reaction temperature, time and frequency of addition, visual color transition, consistency of reaction mixture, reaction time, etc. The processing parameters can be controllable but reactant concentration may not be handled without statistical approaches. The present design enumerates the statistical screening of concentration of oxidizing agent and precursor size on the particle size, PDI, I$_{39}$/I$_{14}$ ratio of GO nanosheets. The critical factors were selected as the volume of sulphuric acid, the concentration of potassium permanganate (numerical factor), and Graphite
size (categorical factor) at 2 levels. One-way ANOVA (analysis of variance) and paired t-test provide the statistical significance of the hypothesis (p-value < 0.05). A significance level of selected factors and response estimated from t-value and p-value.

3. Result and discussion

3.1. Optimization of oxidation process using statistical design

The experimental optimization was performed using $2^3$ full factorial design methodology. The full factorial design analysis provides a complete interaction and statistical verification for the synthesis of GO nanosheets.

The probability plot represented in figure 1(a) shows interaction of all variables at 95% confidence interval (CI). Probability plot analyses the responses at middle level and means of all variables were depicted in table 2.

The average responses were estimated for particle size, PDI and ID/IG ratio using cube plot as represented in figures 1(b)–(d) respectively. The cube plot represents the independent variable is at high level with speciating interaction at the corner points.

The present design elucidates the interaction between the selected numerical factor (chemical oxidizing agents) level and the categorical factor (graphite size). The factorial coefficient table suggests that the p-value was

| Table 2. Probability distribution of variables. |
|-----------------|----------------|-------------|-------|-----|-----|-----|
| Variable         | Graphite size ($\mu$) | Mean      | St Dev | N   | AD  | P   |
| Sulphuric acid (ml) | 8               | 68.5       | 7.506  | 4   | 0.576 | 0.047 |
| Sulphuric acid (ml) | 10              | 68.3       | 7.506  | 4   | 0.576 | 0.047 |
| Potassium Permanganate (g) | 8               | 7.75       | 1.443  | 4   | 0.576 | 0.047 |
| Potassium Permanganate (g) | 10              | 7.75       | 1.443  | 4   | 0.576 | 0.047 |
| Particle size (nm) | 8               | 539        | 298.3  | 4   | 0.173 | 0.807 |
| Particle size (nm) | 10              | 770        | 240.4  | 4   | 0.173 | 0.808 |
| PDI              | 8               | 0.499      | 0.193  | 4   | 0.160 | 0.855 |
| PDI              | 10              | 0.645      | 0.177  | 4   | 0.165 | 0.836 |
| $I_G/I_C$ Ratio  | 8               | 0.7        | 0.240  | 4   | 0.217 | 0.617 |
| $I_G/I_C$ Ratio  | 10              | 0.711      | 0.243  | 4   | 0.170 | 0.821 |
less than 0.05 and assumes the 95% confidence level. The factor analyzed using MINITAB were significant with their respective response.

The standard deviation (SD) of residuals for all 3 responses were analyzed and tested with independent variables indicates strong and prominent interaction. The concentration of reactant has direct effect over their respective response.

From the factorial formation of GO. From the statistical investigation, categorical factor (graphite size) has a less variable effect on the response $I_D/I_G$ ratio. While Relative SD (RSD) represents the variability in the response with the model terms. The RSD was recorded for particle size (98.12%), PDI (99.38%), $I_D/I_G$ ratio (97.47%) respectively, which provides the significance of model terms. The percentage above 80% were perfectly suited for model terms and the balance 5% response was maybe insignificant observed during statistical analysis [14]. The 5% RSD (predicted) was accounted for covariant observed during synthesis. The insignificant values like the effect of graphite size on $I_D/I_G$ ratio were not reflected in model terms and removed (as represented in figure S1 (available online at stacks.iop.org/MRX/8/075602/mmedia) supplementary data) to minimize errors.

$$Y_1 = 3074 + 115.5X_1 - 16.81X_2 - 163.6X_3$$

$$Y_2 = 2.2878 + 0.07275X_1 - 0.01258X_2 - 0.11020X_3$$

$$Y_3 = -1.447 + 0.01485X_2 + 0.1466X_3$$

The regression equations for all output responses denoted as Particle size ($Y_1$), PDI ($Y_2$), and $I_D/I_G$ ratio ($Y_3$) were represented in equations (1)–(3) respectively. Generalized regression equations and predicted equations highlight the interaction between dependent and independent variables. Along with descriptively analyzed linear coefficients, interaction coefficient, quadratic coefficient and error terms as summarized in supplementary data (equation (S1)). The regression equation was used for analyzing responses at variable levels of factors. The coded factors elucidate, $X_1$— for Graphite size, $X_2$ for the volume of sulphuric acid, and $X_3$ for the concentration of potassium permanganate respectively. The regression equation provides the positive or negative effect of variants on given response. The $X_1$ has a positive effect on response terms. The $X_2$ and $X_3$ may show a negative effect on particle size and PDI as reactant concentration increases with decreasing response values. The $X_1$ has minimal effect on response $I_D/I_G$ ratio, while $X_2$ and $X_3$ show the positive effect as increasing oxidation reaction leads to increasing defect and oxygen-containing functionality [23]. From the factorial regression analysis, Graphite precursor size has a larger effect on response particle size, PDI of oxidized GO nanosheets. With simultaneous interaction of numerical factors elucidate the potential oxidizing characteristics of potassium permanganate on the recorded response. While the $I_D/I_G$ ratio response was measured using Raman spectroscopic analysis reveals, oxidizing reactant concentration majorly affects the $I_D/I_G$ ratio with corresponding Graphite size has minimal effect. The coded coefficient as shown in equation (S1) calculates the numerical values helps to screen the feasibility and correlation between variables. The standard error of coefficient has been calculated and shows equivalent values for error factor. The equivalent values suggest that coefficient estimated was a precise one. The t distribution analytics elucidates the effects are statistically significant with corresponding p-values becomes less than 0.05. The variance inflation factor (VIF) from the model term indicates the multicollinearity between the independent factors. The VIF value was found to be 1.00 and use as diagnostic measure of colinearity. The value 1.00 indicates more reliability of statistical data. The percentage of variation would be lesser and changes the levels for coefficients [24, 25].

The total number of degrees of freedom was calculated using linear regression analysis and analysis of variance (ANOVA). The ANOVA shows p values less than 0.05 with each factor has 2 levels, 8 runs. No unusual observations were recorded from the fits and diagnostics data. The linear regression analysis was found to be good fit for the model term.

The Normal plots responses were predicted and confirm residuals are independent and did not have colinearity in the model term (as represented in figure S1 supplementary data). The normal plot provides an integrated significance of factors A (graphite size), B (Sulphuric acid), and C (potassium permanganate) with the model terms used for statistical analysis. The level of significance was found to be above 95% of the confidence interval for Particle size, PDI, and $I_D/I_G$ ratios (figures S1(a), (d) and (g)) respectively. The Pareto chart of standardized effects shows, factors A, B, and C crossing red dotted lines. In the case of graphite factor in $I_D/I_G$ ratio was omitted by the design due to minimal effect. Residual plots of all the responses were depicted in figure 2 helpful for validation of model terms. Compared to normal probability plot, residual points are close to the straight line indicates residuals have constant variance that no other observations were noted. In residual versus fitted plot shows points were randomly distributed along the side of zero axis. No outliers were observed in the model terms as no faraway residual points were observed.

The Residual order versus observation order suggest the residuals were independent from one another and follow a unique pattern for each response (as depicted in figure 2).

The histogram suggests, the particle size, and PDI has larger variations but the values are within the statistical scheme provided. The histogram of residual is bell-shaped shows the normal distribution. The residual versus observation order shows a specialized pattern in particle size, PDI of GO nanosheets. As the concentration of...
reactant increases, particle size was decreased. The decrease in particle size response up to a certain level denotes an increase in dispersibility of nanosheets in water. The $I_{D}/I_{G}$ response ratio shows zig-zag pattern (figure 2(c)) may correspond to a variable degree of response.

The main effect plot for particle size was depicted in figure S2(a) suggests that the incremental concentration of sulphuric acid and potassium permanganate has a direct effect in lowering the particle size. The concentration

Figure 2. Residual plot for standardized residual responses particle size (a), PDI (b), and $I_{D}/I_{G}$ ratio (c).
of potassium permanganate has the largest effect compared to the volume of sulphuric acid. The categorical factor precursor size of graphite may be minimal but important to consider with relevance to achieve the lowest size of synthesized GO nanosheets.

The interaction plot of factors and particle size response was predicted in figure S2(b). The interaction between graphite precursor and sulphuric acid (volume) has minimal interactive effects. The particle size has been lowered due to stronger oxidation amongst sulphuric acid and potassium permanganate produces nitrous acid. The direct variabilities in concentration lead to a reduction in particle size. While the volume of sulphuric acid and potassium permanganate succinctly shows the stronger effect as production of nitrous acid was enough controlled reaction condition. The increase in concentration leads to more interaction and able to convert graphite into GO. The conversion of graphite flakes to GO nanosheets is prominently evident with the stronger oxidation reaction between sulphuric acid and potassium permanganate.

Figure S2(c) notifies the model graph for changes in the PDI value with respective changes in the reactant concentration. The model terms suggest the increasing concentration in numerical factor values decreases the PDI. The case of categorical factor has a slightly different effect compared to numerical factor as presented in figure S2(d). The variable effect generated by factors and their interactive effects was significantly different in the case of response PDI. The residual values were slightly higher in the PDI compared with other responses. The lowest precursor size (8μ) has drawn more attention towards response polydispersity. The distribution of factor concentration with relative increments suggests uniform particle distribution. The interactive modularity was suggested to be more as numerical factor concentration increases. The statistical data suggest that lower precursor size leads to the production of uniform size nanosheet after oxidation reaction as represented in the interaction plot (figure S2(d)). Furthermore, the interaction between sulphuric acid and potassium permanganate was highly effective in the synthesis of least and uniforms nanosheets. The constraints were mostly related to the concentration of the oxidizing agent. The stronger oxidation leads to embedded oxygen-containing functionality and represents an increase in aqueous dispersibility. The dispersibility and uniform distribution of nanosheets in aqueous media were strongly assessed from PDI analysis.

The quantitative estimation of ID/IG ratio provides the extent of oxidation reaction and formation of nanosheets. The model graph shows direct interaction between selected factors and response ID/IG ratio as depicted in figure S2(e). The precursor size has very less effect on the defect ratio. The level of oxidation did not change even after precursor size increases or decreases. The Graphite precursor size was screen as a categorical factor only for measuring the differences in the response (particle size). The volume of sulphuric acid has less effect compared to the concentration of potassium permanganate. The major effect was observed at a higher concentration above 1 (coded value). The Raman analysis suggests the change in concentration beyond 1 (coded value) may lead to a direct reduction of ID/IG ratio and formation of rGO. Figure S2(f) shows an interaction plot for the effect of precursor size on degree of oxidation (measured as response ID/IG ratio). If graphite size (10 microns) is larger then concentration required for optimum oxidation would be higher and vice versa for lower size graphite. The optimal concentration requires for optimum oxidation was up to 10 g for larger size graphite. If concentration of oxidizing agent is above 10 g, graphite starts reducing and converted directly to rGO. At lowest concentration of oxidizing agent about 8 g provide sufficient oxidation for larger size graphite. The concentration ratios with relative correlation was estimated from the interaction plot.
The volume of sulphuric acid has a minimal effect on the $I_D/I_G$ ratio with a simultaneous concentration of potassium permanganate provide the prominent effect. The catalyzing ability and reaction between sulphuric acid and potassium permanganate in a controlled environment possibly embed the transition. The grafting density of oxygen-containing functional groups was increased as reactant concentration increases up to optimum level. The higher concentration of potassium permanganate continues to react with sulphuric acid and generates nitrous acid. In reaction mixture if graphite amount is limited then it may start reducing itself after crossing optimum level of oxidation. It is impossible to control the reaction if additional amount of potassium permanganate available. The factorial design elucidates the optimum level of oxidizing agent to obtain small size GO nanosheets.

Design space elucidates the possible variables and statistical response using contour and surface plots. The plot of particle size versus potassium permanganate with respective sulphuric acid on the $Z$-axis is represented in Figure 3(a). The Graph shows variables in the relative concentration of sulphuric acid and potassium permanganate leads to changes in the response.

The light color indicates design space for variables to achieve the least particle size. The chemical oxidation between reactants catalyzes the graphite which able to generate defects and responsible for the conversion into nanosheets.

The design space was also highlighted for PDI and $I_D/I_G$ ratio in figures 3(c) and (e). A similar indicator was represented from surface plots visualizes the high peaks and trough or smooth area as shown in figures 3(b), (d), and (f). The particle size and PDI have major variabilities compared to quantitative response ($I_D/I_G$ ratio). The middle-level points (design space) show uniform response suggest that small changes in concentration of reactant did not reflect larges changes in $I_D/I_G$ ratio. The larger concentration difference may provide small variations in $I_D/I_G$ ratio.

3.1.1. Response optimizer
Response optimization was estimated using the statistical factorial approach. The standard limits were set for Minimum PDI, particle size, and maximum $I_D/I_G$ ratios suggest two categorical optimizations for 8 Micron and 10-micron graphite individually. From factorial design, we have selected only 8 micro-size graphite for the optimized batch synthesis of GO nanosheets. Figure 4 represents the optimized response consist of 75 ml of sulphuric acid and 9 g of potassium permanganate and all the covariates were set to be constant. The experiments performed in triplicates and analysis were conducted The observed values and predicted values are within the range of confidence interval which is analyzed in further characterization.
Table 3 shows correlation between selected range variables for synthesis and statistical outcome. The graph shows desirability at 0.9753 at given set of variable settings. The outcome of given settings provides response values. The experiments conducted for given concentration provide similar outcomes verifies the statistical data.

3.2. Visual quantification of the oxidation reaction

Visual changes in the reaction estimate qualitative variations were recorded in terms of color, consistency, etc. The first observation was recorded after a complete oxidation reaction between KMnO₄ and H₂SO₄ is consistency and color of reaction. The consistency of the reaction mixture was changed to viscous with drastic color change was noted. At optimum reaction condition the color changes to dark brown as represented in figure 5(a). While the golden-yellow coloration has appeared after the addition of hydrogen peroxide as represented in figure 5(b) preliminary confirm the optimum level of oxidation.

| Variable                        | Setting | Response     | Fit   | SE Fit | 95% upper confidence bound | 95% upper prediction bound |
|---------------------------------|---------|--------------|-------|--------|----------------------------|----------------------------|
| Graphite Size (μ)               | 8       | I₀/I₇ Ratio  | 0.985 | 0.0258 | 1.0376                     | 1.0852                     |
| Sulphuric Acid (ml)             | 75      | PDI          | 0.2800| 0.0139 | 0.3096                     | 0.3312                     |
| Potassium Permanganate (g)      | 9       | Particle size| 225.2 | 35.8   | 301.7                      | 357.6                      |

Figure 5. Visual color observation of optimized reactants during GO nanosheet synthesis, (a) after complete oxidation, (b) after addition of hydrogen peroxide.

Figure 6. UV–vis Spectra of GO1, GO2, GO3, and nGOGF.
The addition of hydrogen peroxide consumes all the Mn$^{2+}$ ions with possibly stops further oxidation. The addition of H$_2$O$_2$ helps to graft oxygen-containing functional groups on GO nanosheet surfaces. The H$_2$O$_2$ addition neutralizes the efficiency of the reactant. The addition of a higher concentration (above 10 g) of KMnO$_4$, starts the formation of rGO with a relative concentration of H$_2$SO$_4$ (75 ml). The color observations were noted after the addition of H$_2$O$_2$ forms dark brown color that merges to black color on storage.

### 3.3. Ultra violet visible spectroscopy (UV–vis)

UV–vis spectra of synthesized GO nanosheets were depicted in figure 6 recorded at variable reactant concentration. The UV–vis spectroscopy preliminary confirms the complete oxidation of GO from graphite precursor. At a higher concentration of the oxidizing agent, KMnO$_4$ accelerates the reaction and converts GO into rGO. The reactant concentration above 11 g (KMnO$_4$) represents the disappearance of shoulder peak (300 nm) and absorbance maxima were shifted to 258 nm. The GO1 is the complete reduced form of graphitized carbon. At a middle concentration of reactant between 9 to 10 g of KMnO$_4$ transition in the oxidation process was observed. The 3 absorbance peaks were observed at 230, 259, and 300 nm [26].

The GO2 represents the complete oxidation with a slight reduction in the GO depicts the removal of oxygen-containing functional groups. The intensity of absorbance maxima at 230 was trying to disappear and the formation of a new peak at 259 nm represents the effect of concentration of oxidizing agent on the formation of GO. The absorbance spectra of GO nanosheets in DW dispersion shows two absorbance maxima at optimum reaction condition. The GO3 spectra were optimized nanosheets showing characteristics absorbance peaks at 230 nm and corresponding shoulder peak appeared at 310–330 nm. The absorbance maxima at 230 emerge due to aromatic bonding between π–π$^*$ (C–C bond) transition, whereas the shoulder peak at 300 nm shows n–π$^*$ (C = O bond) transition [27].

The nGOGF spectrum was represented in figure 6 preliminary shows the passive loading of GF on GO nanosheets. The maximum absorbance peak at 259 nm was quantified for the presence of GF within GO nanosheets. The absorbance maxima ($\lambda_{max}$) of pure GF was observed as 254 nm [28]. The $\lambda_{max}$ was slightly shifted to the right-hand side may be due to the strong interaction between GF nanocrystals and GO nanosheets. The peak intensity increases and broadens due to the accumulation of absorbance maxima from GO nanosheets and GF. The shoulder peak intensity at 343 nm was decreased slightly may be due to strong π–π$^*$ stacking interaction. The GO shoulder peak appeared at 300 nm was shifted towards the right-hand side of the spectrum (343 nm) (highlighted with blue arrow).

UV–vis spectra of pure GF was depicted in figure S3 shows, $\lambda_{max}$ at 250 nm with shoulder peak observed at 335 nm.

**Figure 7. FTIR Spectra of GO (a), GF (b), and nGOGF (c).**
3.4. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectrum of optimized GO nanosheets was represented in figure 7(a). The vibrational frequencies of oxidized carbon allotropes were comparatively evaluated from the earlier reports. The small peak observed at 2783 cm\(^{-1}\) corresponds to sp\(^2\) C–H stretching with the long stretching broad peak at 3267 cm\(^{-1}\) corresponds to the presence of –OH stretching vibration. A strong peak at 1720 cm\(^{-1}\) and 1683 cm\(^{-1}\) shows the presence of carbonyl carbon (C=O) and C=C stretching vibration. The strong peak corresponds to 1610 cm\(^{-1}\) shows C=C stretching. The small peak observed at 1504 cm\(^{-1}\) and 1469 cm\(^{-1}\) shows the presence of C–H and OH bending vibrational frequencies that emerges from oxygen-containing functionalities. The peak observed around 1261 cm\(^{-1}\) corresponds to C–O stretching and 856 cm\(^{-1}\) shows C–C bending respectively. The bond stretching shows successful oxidation of GO as verified from the literature [29, 30].

The FTIR spectra of pure GF were depicted in figure 7(b) shows prominent peak intensities preliminary identifies the supplier provided in pure form. Two strong bands observed at 3520 cm\(^{-1}\), 1302 cm\(^{-1}\) shows the presence of N–H and OH stretch respectively. The low intense peak observed at 3039 cm\(^{-1}\) shows the presence of CH\(_2\)–CH\(_2\). A small intense peak that emerges at 1712 cm\(^{-1}\) shows N–H bending vibration [31]. The intense peak arises at 1631 cm\(^{-1}\), 1566 cm\(^{-1}\) assigned for vibrational frequencies of C=O, C=C and CH=CH (aryl) respectively. Strong bands observed near 1467 cm\(^{-1}\) and 1390 cm\(^{-1}\) shows the presence of OH bending vibration. The carbonyl functional stretching observed at 1141 cm\(^{-1}\) (C=O) with addition peaks for 1033 cm\(^{-1}\) (C–F) and 775 cm\(^{-1}\) (C–Cl) respectively [28].

Fabricated GO nanosheet loaded GF nanocrystalline drug was assessed using FTIR spectroscopy and vibrational frequencies were represented in figure 7(c). Due to the presence of common functional groups vibrational frequencies may overlap, for representation and analysis only identified frequencies were represented. Strong intense peaks observed at 3448 cm\(^{-1}\), 3373 cm\(^{-1}\), 1726 cm\(^{-1}\) correspond to the presence of N–H stretch, OH stretch, and N–H bending vibrations respectively. Two strong peaks appear at 2906 cm\(^{-1}\), 2835 cm\(^{-1}\) shows CH–CH stretching vibration represents strong \(\pi\)-\(\pi\) interaction between aromatic ring structure of GF and GO nanosheets. The peaks observed at 1600 cm\(^{-1}\) and 1548 cm\(^{-1}\) shows common frequency overlap from C=O, C=C, CH=CH stretching intensity of GF and GO nanosheets. A strong bending vibration observes at 1410 cm\(^{-1}\) due to carboxylic OH. The peaks around 1047 cm\(^{-1}\) and 844 cm\(^{-1}\) confirm the loading of GF within internal layers of GO nanosheets (highlighted with red color) [32]. The FTIR analysis partly confirms the successful encapsulation of GF.

3.5. Particle size and zeta potential

The hydrodynamic diameter of optimized GO nanosheets was represented in figure 8. Dynamic light scattering predicts the approximate size of the nanosized carrier along with the PDI value. The GO nanosheets were synthesized from the least size graphite precursor with 8-micron size. The optimum concentration of reactant could reduce the size up to 220.8 ± 0.65 nm with PDI 0.290 ± 0.05. The surface charge on the synthesized GO nanosheet was determined by zeta potential measurement. The synthesized GO nanosheets have a −63 ± 3.1 mV surface charge with 0.1135 mS cm\(^{-1}\) conductivity shows stable dispersion in water for a longer period (>72 h) [30].
The fabricated nGOGF has a slightly increased particle size of about 237.9 ± 0.45 nm with decreasing polydispersity (0.287 ± 0.075) as represented in figure 8. After encapsulation of GF possibly hydrodynamic diameter of particles may be increased with decreasing dispersibility in solution. The quantitative value for zeta potential was decreased for nGOGF and found to be $-33.34 \pm 4.5$ mV with conductivity $0.0777$ mS cm$^{-1}$. The change in physical stability was quantified by comparing the zeta potentials values of GO and nGOGF. A decrease in the stability may be due to the encapsulation of GF nanocrystals within the interlayer spacing of GO nanosheets.

3.6. X-ray diffraction analysis (XRD)
The crystalline structure of synthesized GO nanosheets was depicted in figure 9 representing complete oxidation from graphite precursors. The GO nanosheets spectra show prominent peaks at 2θ angle 11.56°, 22.46°, 38.58°, 42.80°. The miller indices of the designated peaks were appeared as (1 1 1), (2 2 2), (1 0 0), (2 0 0) respectively (figure 9). The peaks at 11.56° and 22.46° represent complete oxidation of graphite to GO. The crystalline intensity was compared with the JCPDS-ICDD database library [33].

The GF was available in crystalline form with triclinic crystallites represents the strong 2θ observed at 30.36°, 35.72°, 43.40°, 53.80° with representative miller indices found to be (1 0 0), (1 1 0), (1 0 0), and (1 1 0) respectively as extracted from figure 9. From the given data GF was found within P1 space groups in triclinic crystallite structure and compared with reference data [28]. The high-intensity peaks define the uniforms stable crystals were present with additional peaks at 57.37° (0 0 0).
The nGOGF shows highly crystalline spikes after analysis as represented in figure 9. The individual intensity for graphitized carbon was shifted slightly representing similar crystallite size at 10.95°, 11.59°, 13.16°, 17.05° with (1 0 0), 20.77° with (1 1 0), 26.87° with (1 1 1), 29.49° with (2 0 0), 31.56° with (2 0 0), 35.44° with (2 1 0), 42.70° with (2 2 0), 46.93° with (3 0 0), 49.75° with (3 1 0), 56.41° with (3 2 0) respectively [33, 34]. The π-π* stacking interaction splits graphitized carbon intensities into two individual spectra at 10.94° and 11.59° having (1 0 0) planes. The conversion of the nanocrystalline drug may increase the number of crystalline peaks but crystallite size was similar as verified by measuring miller indices. The nanocrystalline GF encapsulated into GO nanosheets may empower the desirable crystallinity. The observed intensity decreased slightly compared with pure GF intensities. The crystallite structure of GF did not change after the sonication process and shows distinct peaks at structured positions to form a triclinic crystal plane. The XRD analysis entails the GF was encapsulated within interlayer spaces of GO nanosheets with nanocrystal size less than 2.4 nm as calculated using Debye-Scherrer equation.

3.7. Raman spectroscopy
A disordered defect in the allotropic forms of carbon was verified using Raman spectroscopic analysis. A Raman spectrum of an optimized batch of GO nanosheets was depicted in figure 10. The peak intensity increased after oxidation in presence of KMnO₄ with the broadening of the D band. The G peak intensity was also increased to a high value compared to the D band. At a higher concentration of KMnO₄ (above 9 g) intensity of the G band decreases with a slight increase in D band intensity represents the formation of reduced GO [35]. The optimum concentration of reactants leads to the formation of an oxidized form of graphene with a prominent intensity spectrum of G (1538 cm⁻¹) and D (1390 cm⁻¹) bands. The optimized concentration shows a disordered ratio I_D/I_G in between 0.9 to 1.09. We have considered I_D/I_G ratio is a critical quality attribute in the present investigation. The G band emerges due to C–C band stretch whereas the D band was showing defects generated during the oxidation process in the graphite precursor. The second-order overtone spectra appear as 2D peaks around 2709 cm⁻¹ shows the number of graphene layers. The appearance
of 2D peaks in graphitized carbon is independent of the presence or absence of D band intensity. The peak appears at 3039 cm$^{-1}$ was due to the D + G band shows defect activation after oxidation in presence of KMnO$_4$ [6, 31].

A Raman spectrum of GF was depicted in figure 10. The vibration band of quinazoline was ascribed at 1325 cm$^{-1}$ assigned for CH$_2$ scissoring vibration. The vibrational modes of floro-benzene ring was appears at 1613 cm$^{-1}$ [36]. The intense band appears at 2746 cm$^{-1}$ was due to the presence of 4Methyl morpholine shows association CH$_2$ vibrational intensity in hydrogen bonded interaction. The C-N stretching vibrations appeared at 1017 cm$^{-1}$ emerges from aromatic amines. The small peak appears between 200–727 cm$^{-1}$ designated for the presence of C–X (C–Cl, C–F) stretching modes. The band appears at 2746 cm$^{-1}$ assigned for asymmetric stretching vibration mode [37].

Figure 10 represents the Raman intensity spectra of fabricated nGOGF. The change in the vibrational intensities of GO nanosheets suggest the encapsulation of GF. The vibrational frequency assigned for CH$_2$ twisting and wagging emerges from quinazoline and morpholine ring band were merged with D and G spectral intensities of GO. The wavenumber was shifted slightly to higher value at G band position. The transition was observed from 1538 cm$^{-1}$ to 1610 cm$^{-1}$, suggest the strongest interaction between GF nanocrystals and GO. The band intensity at 2D and D+G band were increased slightly may be due to strong aromatic interaction at 2745 cm$^{-1}$ and 3046 cm$^{-1}$ respectively. The increase in the 2D peak intensity represents an increase in interlayer coupling and spacing. The interlayer spacing increases due to more encapsulation of GF nanocrystals and cavitation energy [38]. The encapsulation of GF possesses strong interaction of haloatoms, the vibrational spectra assigned for C–X (C–Cl and C–F) at 623 cm$^{-1}$ position shows increase in Raman intensity. From the above vibrational intensity shifts the nGOGF was successfully encapsulated with nanocrystals and possess strong interaction.

3.8. Scanning electron microscopy (SEM)/elemental analysis (EDX)

Morphological and structural investigation of an optimized batch of GO nanosheets was depicted in figures 11(a) and (b). The scanning electron microscopic images shows exfoliated nanosheets were separated during the oxidation process and flakes were aggregated during drying. The solid specimen was directly placed on carbon tape possibly forms the aggregated structure of GO nanosheets. The structural distinction suggests the nanoflakes were separated during oxidation reaction and interlayer spacing was visible in figure 11(b). The simple sonication process can remove the aggregates and individual nanosheets were separated [39].
The elemental concentration of the optimized batch shows a higher concentration of oxygen and carbon as shown in figure 11(e). The atomic weight percentage of carbon and oxygen was found to be 61.16 and 38.84% respectively. The elemental graph did not show any impurity during estimation suggest that process is viable for the synthesis of the nanocarrier. The EDX spectrum was in line with supportive Raman intensities verifying complete oxidation of graphite and embedded oxygen-containing functional groups on surfaces [26].
Figures 11(c) and (d) shows structural features of fabricated nGOGF and morphological analysis for encapsulated GF. The investigation shows GF was deposited inside the interlaying spacing of GO nanosheets, suggest a strong interaction between GF nanocrystals. The probe sonication approach was able to convert GF nanocrystals and deposited them on the surface of GO nanosheets. The interlayer distance between GO nanosheets was increased after processing and hydrodynamic diameter reflects an increase in particle size of nGOGF. The 2D value Raman spectra were also shown to decreases intensity suggest that GF nanocrystals were deposited inside the layers of GO nanosheets as verified by morphological observations \[35, 40\].

The elemental observation of nGOGF was recorded and depicted in figure 11(f). The EDX spectrum suggests presence of Carbon (70.19%), Oxygen (29.16%), Nitrogen (0.30%), Chlorine (0.10%) and Fluorine (0.19%) at variable atomic percentages. The data suggest that The presence of N, Cl, and F in the spectrum of nGOGF suggest, GF was successfully loaded with GO nanosheets. The Oxygen percentage was decreased with increasing carbon percentage shows strong aromatic interaction between GO and GF.

### 3.9. High-resolution transmission electron microscopy (HRTEM)

The internal structure of GO nanosheets was depicted in figures 12(a) and (b), which clearly shows small flakes were separated during the oxidation process and nanosized sheets were formed. The particle size analysis using dynamic light scattering reveals the size of GO was within nanosized range. The hydrodynamic diameter of GO nanosheets was observed around 220 nm and the internal structure was of GO nanosheet successfully confirmed. The transmission electron microscopic image of exfoliated GO nanosheet was represented in figure 12(b) shows uniformed layer get separated after the exfoliation process. The wrinkled surface generated after the treatment of oxidizing agent shows the formation of an oxygen-containing surface on GO nanosheets \[40, 41\].

Figures 12(c) and (d) shows the internal structure of fabricated nGOGF suggests the sonication method extensively reduces the size of GO nanosheet and grafts GF successfully. The small dots/pits represented on the wrinkled GO nanosheet were encapsulated with GF nanocrystals. The nanocrystalline nature of GF was verified from the XRD spectra with a size calculated about 2.4 nm using the Debye–Scherrer equation. Figure 12(d) shows GF nanocrystals deposited on the surface of GO nanosheet with a size less than 2 nm \[34, 41\].

### 3.10. X-ray photoelectron spectroscopy (XPS)

Complete oxidation states and functional groups of synthesized GO nanosheets were analyzed further using x-ray photoelectron spectroscopy as represented in figure 13(a). Figure 13(b) shows carbon 1 s spectra having 3 peaks with respective binding energies was found to be 284, 286, 289 eV. The corresponding \(\pi-\pi^*\) satellite shield with deconvoluted peaks of C–C (0.314), C–O (16.78), and O–C=O (71.87) was established after analysis \[7, 29\].

Figure 13(c) shows the oxygen 1 s region established after the oxidation process of GO nanosheets shows deconvoluted peaks at 529, 531, 532, 533 eV. The binding energies represent the presence of O–C=O, C=O, C–OH and C–O–C respectively. The peak intensities were compared with reported literature, suggest the complete oxidation of GO nanosheet was processed in presence of the oxidizing agent. The hydrophilicity was increased with the sp² zone significantly transformed after oxidation \[42\]. The details of comparative peak assignment, atomic percentage, and binding energy were represented in table 4.

![Figure 14. In-vitro Drug release of pure GF and nGOGF.](image-url)
3.11. In-vitro drug release

Drug release characteristics of nanocrystalline GF from GO nanosheets were represented in figure 14. The comparative evaluation was performed for pure GF to understand the release and dissolution characteristics of nanocrystalline GF\[43\]. The investigation suggests the percent encapsulation efficiency of nanocrystalline GF was found to be 78% and the percent drug loading achieved using the probe sonication method was 43%. The nanocrystalline GF was deposited on the surfaces and interlayer spacing of GO nanosheets as understood from surface morphology. The least crystallite site identified from XRD and TEM analysis may help enhance the dissolution characteristics of GF in phosphate buffer pH 7.4. The GF has very poor solubility in phosphate buffer pH 7.4. We have modulated the process for pure GF and used 2% surfactant. By the use of surfactant is helpful for increasing the solubilization process and permeates across the porous membrane. The addition of surfactant also helps to quantify the GF using spectrophotometric analysis. The GF release up to 51.3% for 8 h may be due to poor solubility characteristics. The nanocrystalline GF releases from GO nanosheet in a very slower manner, an interlayer spacing did not allow instant diffusion of buffer and possibly retard the release. Two-fold increasing dissolution characteristics of nanocrystalline nGOGF were observed compared to pure GF\[44, 45\]. The release of nanocrystals of GF shows 92.5% observed at the end of 8 h.

4. Conclusion

The present investigation highlights the credibility of process factors during the fabrication of nanocarriers for drug delivery. The full factorial design analysis confirms the statistical significance and independent level of model terms. The statistical screening precludes the 95% confidence interval and interaction between factors and responses. The chemical oxidation and size of the precursor appropriately provide a strong response with optimum synthesized GO nanosheets. The variable concentration of reactant can be optimally selected based on desired sizes of nanosheets required. The process screens the possible interaction between reactant and graphite precursor with the least concentration and high productivity. The process successfully creates nanosheets of GO in a controlled environment at the laboratory. The designed process motivates the young scientist to screen for the possible assumptions and simplified way of characterizing GO nanosheets. The conversion of crystalline drug to nanocrystalline drug without change in its phase was very critical. The controlled environment may be useful for the successful preparation of GF nanocrystals and encapsulation within GO nanosheets. The nanocrystalline GF enhances the dissolution characteristics compared with Pure GF and could be a useful alternative in future drug delivery. Without the use of any surfactant, the process feasibly converted GF into nanocrystals was analyzed using high-resolution transmission electron microscopy and XRD. The investigation opens new avenues for understanding design space for the chemical oxidation reaction and designing nanocarrier for drug delivery application. In the future, the designed nanosheet may be used for loading multiple therapeutic agents and biomolecules. The nanosheets effective surface interaction and stability in dispersion media. The synthesized GO nanosheets also act as a substrate for biosensing applications.

Data availability statement

No new data were created or analysed in this study.

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