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Highly enhanced loading quality of curcumin onto carboxylated folate graphene oxide

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Abstract. This research focuses on loading of curcumin (Cur) anticancer drug onto nanocarriers, based on Graphene Oxide (GO), and improvement of loading efficiency. Surface of synthesized GO was modified by Citric Acid (CA) and functionalized by Folic Acid (FA) as a targeting agent. The functionalized GO with CA (CGO) and FA (GO-FA) was analyzed by Fourier transform infrared (FTIR). Furthermore, FA was conjugated to the composite of CGO to prepare a stable and targeted GO. The CGO-FA composite was characterized by FTIR and Scanning Electron Microscopy (SEM) analysis. Thereafter, Cur as a hydrophobic drug was loaded onto GO, CGO, GO-FA, and CGO-FA. The loaded Cur onto GO was characterized by SEM, FTIR, and UV-Vis spectrophotometry. To increase the loading efficiency of Cur, the effects of water and ethanol as solvents and the weight ratios of initial Cur to GO (Cur/GO) were evaluated on the loading efficiency by response surface methodology. The comparison of the loaded drug efficiencies on different carriers demonstrated the maximum loading onto CGO, as compared with the other carriers, in optimal conditions. The optimized condition was characterized by 25.6% of water in solution and 1.66 ratio of Cur/GO to achieve the loading efficiency of 112.5% and 13.5 ratio of loading efficiency/weight of initial Cur, respectively.

1. Introduction

Despite the recent progress in medical sciences, cancer exists today as a big challenge to humanity [1]. In 2012, cancer was one of the main causes of death worldwide. Based on the last studies, it is expected that, by 2020, between 15 and 17 million people will be diagnosed with cancer every year [2]. Therefore, further efforts are surely required to treat cancer.

Graphene Oxide (GO) is one of the most important derivatives of graphene and a suitable choice to use in drug delivery system. The surface area of GO is around 2600 m^2/g which is higher than the surface area of other nano materials used in drug delivery [3]. Thus, the capacity of drug loading onto GO is considerably higher than that of any other nano materials. Efficiency of drugs loading onto GO may increase up to 200%, which is much higher than those of other drug carriers [4]. Nowadays, GO is a promising carrier for anticancer drugs [5,6]. GO is comparable in structure to graphene; however, there is a combination of carbon atoms with sp^2 and sp^3 hybridization in its structure resulting from the presence of oxygen-containing functional groups [7]. Due to its surface chemistry, graphene is strongly hydrophobic and requires surface-active agents. However, contrary to

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graphene, GO is water dispersible due to its oxygen containing functional groups, and it would turn into a colloidal or a stable form [4].

Liu and coworkers pioneered the study on GO for biopharmaceutical applications as an effective nano drug carrier [8]. The new nanocarrier was discovered to deliver aromatic drugs that are insoluble in aqueous solutions into the cells. Zhou et al. suggested that graphene and GO could inhibit the migration and invasion of human breast and prostate cancer cells towards healthy cells [9].

In addition, the research demonstrated that low concentrations of graphene and GO exhibited no considerable toxicity. Based on the studies, increasing solubility and dispersibility of graphene and its derivatives might cause higher biocompatibility. Compared with graphene, GO exhibited more compatibility due to the presence of oxygen containing functional groups. In addition, surface modification of graphene and GO significantly increased their biocompatibility [10]. Similar to graphene, GO has a two-dimensional structure, yet it contains carbonyl, hydroxyl, epoxy, and carbonyl groups (oxygen-containing groups), improving surface easier and more practical. Therefore, in order to make GO more stable in aqueous salt solutions, its surface must be functionalized [4].

Because of its considerable therapeutic properties, Citric Acid (CA) was used as a surface improvement agent to increase biocompatibility and stability of drug carriers. CA contains carboxyl and hydroxyl functional groups that can be conjugated with hydroxyl and carboxyl functional groups of GO by covalent attachment with a simple esterification reaction. Nurunnabi et al. utilized CA for carboxylated Graphene Quantum Dot (GQD) and increased solubility and stability of physiological solutions [11]. Moreover, the carboxylated GQD demonstrated toxicity neither in vitro nor in vivo.

Despite the advantages such as solubility and persistence in blood circulation that many drug carriers possess, their use is limited by their incapability for targeted application in treating cancerous tumors. Wang et al. had Folic Acid (FA) GQD covalently conjugated and used for targeting delivery of Doxorubicin (DOX) anticancer drug [12]. They demonstrated that the loaded drug onto targeted carrier, GQD-FA/DOX, affected target cells and showed no impact on healthy cells. In addition, Zhang et al. had FA and GO conjugated and loaded two anticancer drugs, DOX and CPT, onto GO-FA. This carrier enabled targeted drug delivery with more efficiency [13].

Although Cur has fewer side effects than other anticancer drugs do and does not damage healthy cells, it is insoluble in aqueous solutions [14]. Therefore, slight amounts of this drug are absorbed by cells and the rest are excreted from the body. Moreover, degradation of Cur in neutral and basic pH environments and its sensitivity to light and low bioavailability have limited its therapeutic applications including its use for cancer treatment [15,16]. Therefore, a drug carrier must be used to increase drug solubility in aqueous solutions and improve therapeutic applications. GO is a drug carrier that has satisfactorily resolved the associated problems using Cur as an anticancer drug.

Using a simple mixing method, Some et al. loaded Cur on GO, more extensively oxidized GO, and GQD. They studied the extent of Cur loaded onto these three drug carriers, and found that maximum loading occurred on the GQD, and the degree of loading on the more extensively oxidized GO was higher than that of GO due to the polar reactions of Cur with oxygen containing groups. These researchers concluded that the extent of Cur loaded onto carriers improved with increasing oxygen-containing functional groups on their surfaces. Moreover, their results suggested that the combination of graphene compounds and Cur exhibited considerable anticancer activity [17].

Maity et al. covered the surface of GO with the polymers, including chitosan and dextran, to increase its colloidal stability. They also produced targeted nanocarriers for transferring hydrophilic and hydrophobic drugs (such as Cur) by attaching the carboxyl group in FA to the amino end groups of chitosan. Results of their research indicated that the extent of loading drugs depended on their chemical structures; moreover, those drugs with aromatic rings, such as Cur with functional groups, were more absorbed on the surface of GO, as compared to other drugs [18].

In the present research, CGO, GO-FA, and CGO-FA nanocarriers are produced. Carboxylation with CA has been applied to increase the stability of GO in aqueous solutions, and functionalization with FA has been carried out to develop a targeted drug delivery system. Cur would be loaded onto these nanocarriers using a new and simple method. A mixture of the two solvents of water and ethanol has been used to increase the extent of Cur loading. The statistical method of response surface methodology has been applied to search the significant effect of synthesis parameters and optimization of the conditions.

2. Materials and methods

2.1. Materials

Graphite powder was purchased from Fluka (purity 99 wt.%); sulfuric acid (98 wt.%), orthophosphoric acid (85 wt.%), potassium permanganate (99 wt.%), citric acid (99 wt.%), folic acid (99 wt.%), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (99 wt.%), and N-hydroxysuccinimide (NHS) (99 wt.%) were purchased from Merck; Cur was prepared by soxhlet extraction method with acetone

(86% purity). Hydrogen peroxide (37 wt.%) was purchased from Mojallali.

2.2. Preparation of CGO, GO-FA, and CGO-FA

Graphene Oxide (GO) was synthesized using the previously reported method based on the improved Hummers' method [19-21]. Briefly, a mixture of H_2SO_4/H_3PO_4 (40.5:4.5 ml) was added to graphite powder (1.0 g). $KMnO_4$ (6.5 g) was gradually added during one hour at the controlled temperature below 20°C. The reaction mixture was then heated up to 37 ± 2°C and mixed using a mechanical agitator for two days. Then, the mixture was sonicated (170 W) for 30 min. Thereafter, the reaction mixture was cooled down to ambient temperature. Diluted H_2O_2 was added drop wise to the GO in an ice bath. Then, the mixture was centrifuged (7000 rpm) and washed repeatedly with deionized water and ethanol (96% v).

To prepare CGO, 45 mg of CA was added to the solution of GO in 10 ml of ethanol (1.5 mg/ml) and stirred for five hours at ambient temperature. The solution was then washed several times with deionized water and centrifuged (7000 rpm). Then, CGO was obtained.

To prepare GO-FA, the solution of GO in phosphate buffer saline (pH = 7.4) (5 ml at the concentration of 0.7 mg/ml) containing EDC (96 mg) and NHS (59 mg) was exposed to the reaction for two hours at ambient temperature. FA (10.5 mg) was then added and stirred for 18 hours. The solution was then washed twice with phosphate buffer and four times with deionized water and centrifuged (7000 rpm). To prepare targeted and stable CGO-FA nanocarriers, FA was attached to CGO using the method described to prepare GO-FA.

2.3. Cur loading

The solvent included 80% of water and 20% of ethanol added to 10 ml of a GO solution (at the concentration of 0.5 mg/ml) to load Cur onto GO. Cur at twice the weight of GO was then added to the solution and stirred for three hours at ambient temperature in the dark. The solution was then centrifuged and washed several times with deionized water to remove unloaded Cur from the solution. After centrifugation, Cur rose to the surface of the solution because of its lower weight and was easily removed, and the loaded Cur precipitated together with its carrier. The same loading procedure was followed to load Cur onto CGO, GO-FA, and CGO-FA carriers.

To estimate the degree of loading, after removing the supernatant solution in the last stage of centrifugation, some acetone was added to the remaining sample, and the Cur was rapidly released into the acetone. After an hour, this solution was centrifuged, the supernatant liquid that contained the Cur dissolved in the acetone was removed, its volume was determined, and the released Cur was measured using a visible spectrophotometer. The absorbance at 419 nm was measured with a Unico 2100 visible spectrophotometer to determine the concentration of Cur in a carrier/Cur solution. The Cur loading efficiency was calculated based on the correlation: Loading efficiency (%) = (weight of loaded drug/Weight of carriers)*100.

3. Statistical experimental design

Response surface method (RSM) was employed to design experiments for the purpose of optimizing the parameters that influence Cur loading onto GO; in addition, the results of these experiments were analyzed using the Design Expert 7.0.0 software. The purpose of designing these experiments is two-fold: to obtain a suitable weight ratio of the drug and the carrier and, thereafter, to determine the optimum ethanol concentration for achieving higher loading efficiency with lower initial quantities of Cur.

The Rotatable Central Composite Design (RCCD) was employed among different procedures of RSM. The weight ratio of Cur to GO (Cur/GO) (X_1) and percentage of ethanol in the solvent (water-ethanol) (X_2) were selected as the effective parameters, and parameters such as concentration of GO and duration of the experiment were kept constant. Five levels ($-\alpha$, –1, 0, +1, $+\alpha$) were considered for each parameter, and $\alpha = 2$ was selected as the distance between the axial points and the center point in this method. Generally, 13 experiments, including five replications, at the central point, were conducted and, based on the reviews that were carried out, the two efficiency parameters of Cur loading on GO (Y_1) and the ratio of loading efficiency to initial Cur weight (Y_2) were considered in this experimental design.

4. Results and discussion

4.1. Characterization of CGO, GO-FA, and CGO-FA

The Fourier transform infrared (FTIR) spectra were measured with a Perkin-Elmer spectrometer in the frequency range of 400-4000 cm^{-1}. The FTIR spectrum confirmed the carboxylation of GO. In Figure 1, the peaks at 2850 cm^{-1} and 2923 cm^{-1} are those of the C-H tensile bond in alkane. Furthermore, the displacement of the hydroxyl (O-H) peak from 3425 cm^{-1} (the GO spectrum in Figure 1) to 3405 cm^{-1} could also represent attachment of CA to GO through ester linkages. Moreover, the peaks at 1733 cm^{-1} and 1230 cm^{-1}, which are those of C=O and C-O of carboxyl and ester, were also formed, and all these peaks indicate the attachment of CA to GO.

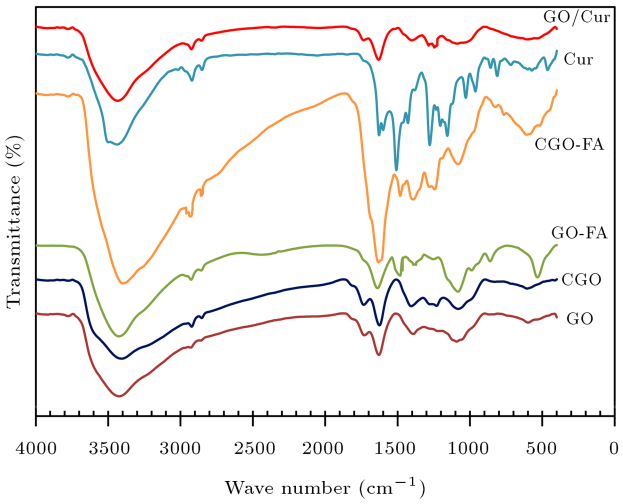

Figure 1. FTIR spectra of GO, CGO, GO-FA, CGO-FA, Cur, and GO/Cur.

In the FTIR spectrum of GO-FA (Figure 1), the peaks at 2926 cm^{-1} and 2850 cm^{-1}, related to the C-H tensile bond in alkane, appeared. Furthermore, a peak observed at 1480 cm^{-1} related to the pterin ring, a peak at 1250 cm^{-1} belonged to the C-N bond of the pterin ring, and a peak at 1630 cm^{-1} related to the amide group, while the peak belonging to the primary amino group was deleted. Therefore, it could be concluded that FA was attached to GO. As shown in Figure 1, the peak associated with the primary amino group in CGO-FA was also deleted; in addition, those belonging to the pterin ring and the C-N bond were formed at 1480 cm^{-1} and 1248 cm^{-1}, respectively. Therefore, surely, FA was attached to GO.

Scanning Electron Microscope (SEM) was used to identify the surface morphology of GO and CGO-FA. SEM images were performed with a Hitachi-S4160 microscope operating at 30 kV. Thin and wrinkled layers can be seen in the SEM image of GO (Figure 2(a)). Moreover, the SEM image of CGO-FA shows the attachment of CA and FA to GO, thus disrupting the orderly structure of GO (Figure 2(b)).

To examine stability, two solutions of CGO in PBS (pH 7.4) and GO in PBS were prepared with equal concentrations (0.5 mg/ml) and sonicated. As shown in Figure 3, after 18 hours, the GO solution precipitated, while the CGO solution was still stable.

Figure 4 presents the mechanism of synthesized CGO-FA, GO-FA, and CGO. GO was synthesized using the improved method; then, it was converted into carboxylated GO through attaching CA. In this way, the esterification reaction took place between the carboxyl functional groups in the structure of CA and the hydroxyl functional groups in GO; thus, a covalent bond was formed between CA and GO. The hydrophilic groups of CA make carboxylated graphene oxide (CGO) soluble and stable in aqueous phase media. The primary amino groups in FA also reacted with the carboxyl groups in GO and CGO in the

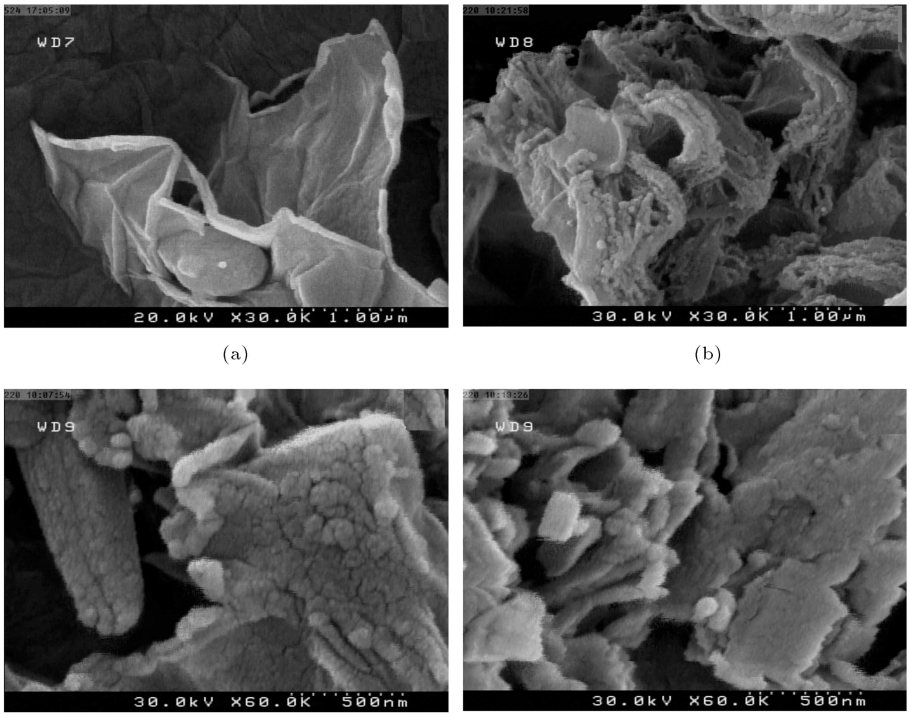

Figure 2. SEM images: (a) GO, (b) CGO-FA, (c) GO/Cur, and (d) CGO-FA/Cur.

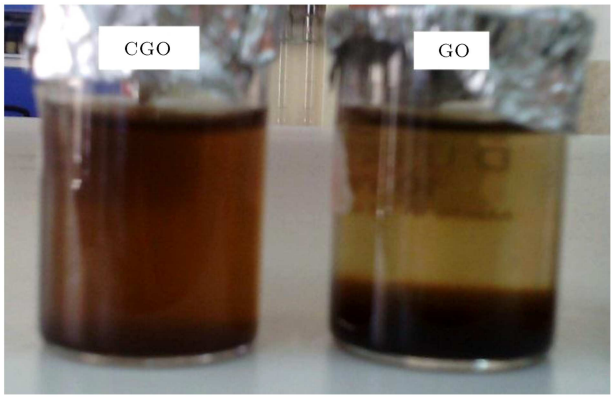

Figure 3. Images of GO and colloidal CGO in PBS after 18 hours.

presence of EDC, and Sulfo-NHS, GO-FA, and GCO-FA were synthesized through the formation of amide bonds.

4.2. Loading study of curcumin

Loading Cur on the various carriers was performed via a simple method of using a stirrer to mix the colloidal carriers with Cur. Cur has phenolic hydroxyl functional groups in its structure that can form hydrogen bonds with hydroxyl groups and carboxyl groups in GO, GO-FA, and CGO-FA in this stage. Moreover, the formation of π-π bonds between the benzene rings of GO and Cur would cause Cur absorption on the surface of the carriers (Figure 5).

In the FTIR spectrum of the Cur loading on GO (GO/Cur) (Figure 1), the peak related to the O-H bond appears at 3433 cm^{-1}, which shows a displacement in relation to the hydroxyl peak in GO (at 3425 cm^{-1}). Therefore, hydrogen bonds were formed. Furthermore, the peaks related to the C-H bond in the alkane group were also formed at 2880 cm^{-1} and 2924 cm^{-1}. Moreover, the characteristic peak of Cur (Figure 1) that is related to the C-O bond attached to the methyl group (the C-O methyl bond) was observed at 1258 cm^{-1} [22].

Contrary to the SEM image of GO (Figure 2(a)), there are protuberances and roughness with the average size of about 60 nm on the surfaces and edges in the SEM image of GO/Cur (Figure 2(c)) and also in that of CGO-FA/Cur (Figure 2(d)). Changes in the morphology of GO and CGO-FA indicated that Cur was physically absorbed on these nanocarriers [17]. Therefore, by using the loading method, Cur came into contact with carriers based on GO and was successfully loaded onto the surface of the carriers through forming hydrogen bonds [17] and π-π stacking [23] on the surface of these carriers in the absence of UV light. The quantity of loaded Cur was estimated by its rapid release into acetone and through measuring this released Cur using the instrument for measuring absorption spectra.

Thereafter, the effects of using two solvents, water and ethanol, were studied on the efficiency of loading Cur onto GO. As shown in Figure 6, loading efficiency improved with reductions of ethanol fraction in the solvent. Cur is insoluble in water, and both the carrier and drug must be completely dissolved in the solvent to form physical bonds. Therefore, ethanol was added as the second solvent to dissolve Cur and bring Cur

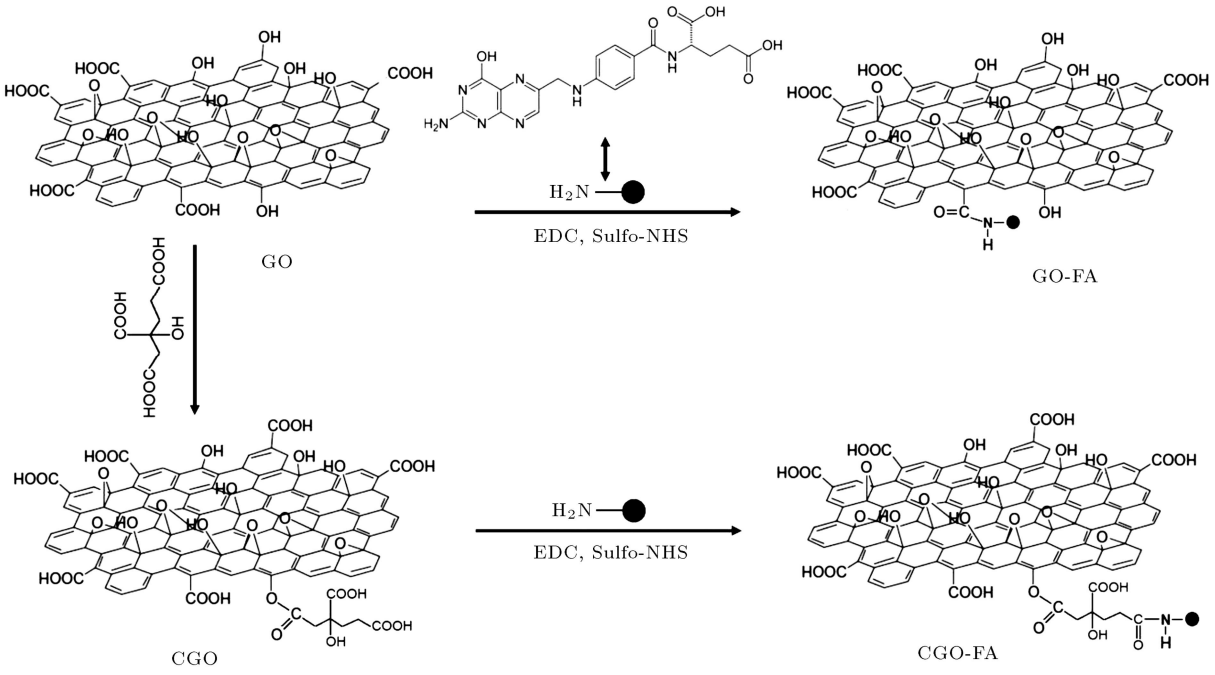

Figure 4. Schematic illustration of the preparation of CGO, GO-FA, and CGO-FA.

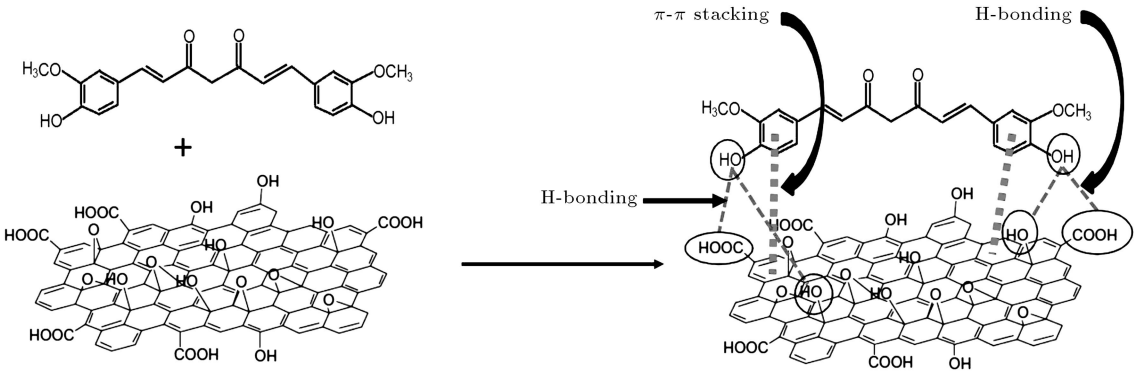

Figure 5. Schematic illustration of loading of curcumin onto graphene oxide.

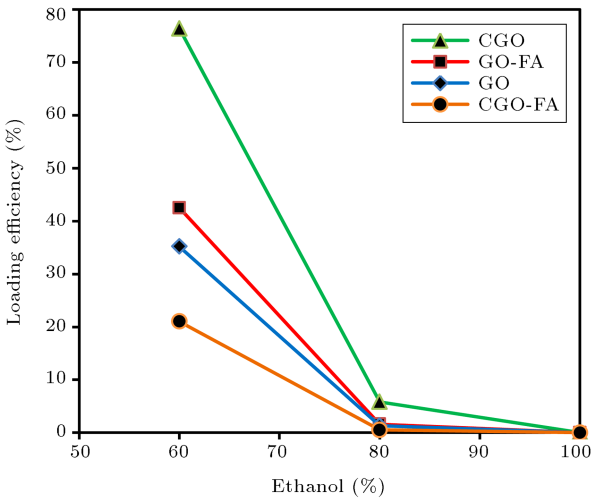

Figure 6. Variation of loading efficiency with ethanol percentage.

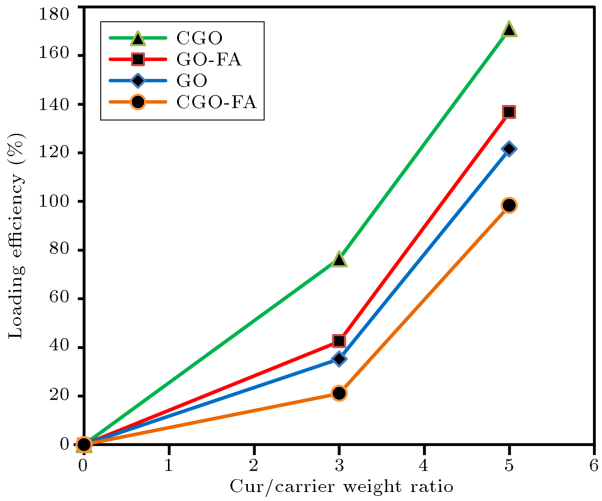

Figure 7. Variation of loading efficiency with Cur/carrier weight ratio.

and the carrier close together. Consequently, physical bonds were formed between Cur and the carrier. Moreover, it is not desirable to have excessive amounts of ethanol as a solvent because Cur is highly soluble in ethanol. If ethanol occupies a large part of the solvent, its molecules completely surround Cur molecules and limit the attachment to the carrier molecules.

The initial concentration of Cur is another factor that influences loading efficiency. Therefore, Cur loading efficiency was determined using various initial amounts at constant carrier concentrations under identical conditions. Figure 7 shows that loading efficiency improves with increasing the weight ratio of Cur and the carrier.

5. Statistical study

Table 1 presents a number of tests on the basis of the experimental design with two responses. The responses were analyzed by analysis of variance (ANOVA) using Design Expert 7.0 based on F test, and the probability values are reported in Table 2. Considering confidence level of 95%, all the factors were significant for the loading efficiency (Y_1) and for the ratio of loading efficiency to initial Cur weight (Y_2).

5.1. Loading efficiency

Considering ANOVA for loading efficiency (Table 2), the quadratic model fits well with the data and suitable coefficients are obtained as follows. It should be noted that the parameters with P values of less than 0.05 are significant and the rest of them are omitted. It can be seen that the main parameters, pairwise intervention of parameters, and one of the squared interaction effects are significant. Moreover, the insignificant lack-of-fit error (P value > 0.05) indicates that the data can be described well by the proposed regression model. Therefore, considering all the main effects in the model and the significant squared and interaction effects, the regression model for efficiency of Cur loading (based on the coded parameters) is written as in Eq. (1):

$$Y_1 = (38 \pm 3.49) + (31.26 \pm 2.42) \times X_1 - (37.35 \pm 2.47) \times X_2 - (33.25 \pm 4.2) \times X_1 \times X_2 + (6.66 \pm 1.75) \times X_1^2 + (10.26 \pm 1.75)$$

Table 1. Central composite design for preparation of Cur loaded onto GO.

Run	Factors (real and coded)		Responses	
	X_1 [a]	X_2 [b]	Y_1 (%) [c]	Y_2 (%/mg) [d]
1	3(0)	60(0)	31.3	2.09
2	4(+1)	80(+1)	0.62	0.031
3	4(+1)	40(−1)	190	9.5
4	5(+2)	60(0)	121.5	4.86
5	3(0)	60(0)	30.8	2.05
6	3(0)	60(0)	32.5	2.17
7	3(0)	60(0)	38.9	2.59
8	3(0)	60(0)	42.4	2.83
9	2(−1)	40(−1)	56.7	2.67
10	2(1)	80(+1)	0.34	0.034
11	3(0)	20(−2)	150	10
12	1(−2)	60(0)	0.75	0.15
13	3(0)	100(+2)	0.59	0.039

[a] X_1: Cur/GO ratio; [b] X_2: ethanol percent (%);

[c] Y_1: loading efficiency (%); [d] Y_2: loading efficiency/initial Cur (%/mg).

Table 2. Analysis of variance for the central composite.

Source	*p*-value	
	Loading efficiency (%)	Loading efficiency (%)/ weight of initial Cur (mg)
Model	< 0.0001	< 0.0001
X_1	< 0.0001	0.0007
X_2	< 0.0001	< 0.0001
X_1X_2	0.0002	0.0191
X_1^2	0.0090	0.6897
X_2^2	0.001	0.0016
$X_1^2X_2$	0.0034	0.0131
Lack of fit	0.0645	0.0476
R^2	0.9792	0.9644

$$\times X_2^2 - (24.08 \pm 5.14) \times X_1^2 \times X_2. \quad (1)$$

5.2. Ratio of loading efficiency to initial Cur

The ratio of loading efficiency to initial Cur amount (Y_2) was analyzed by ANOVA, and it was concluded that the main parameters and interaction effects were significant (Table 2), whereas the square of the ratio of Cur to GO (X_1^2) was not significant (P value $>$ 0.05). Moreover, the lack of fit was not significant with confidence level of 94.7%, concluding that the fitted model would be significant. The regression model for the ratio of loading efficiency to the initial Cur (based on the coded parameters) is presented in Eq. (2):

$$\begin{aligned} Y_1 = {} & (2.55 \pm 0.25) + (1.1 \pm 0.17) \times X_1 \\ & - (2.49 \pm 0.21) \times X_2 - (0.96 \pm 0.3) \times X_1 \\ & \times X_2 + (0.68 \pm 0.13) \times X_2^2 - (1.29 \pm 0.37) \\ & \times X_1^2 \times X_2. \end{aligned} \quad (2)$$

As shown in Figure 8 (a) and (b), the 3D plots of Y_1 and Y_2 versus X_1 and X_2 are presented. Both responses are improved by increasing Cur/Go ratio and decreasing ethanol fraction.

5.3. Optimization

The optimal factors were derived by using the quadratic models to approach the maximum ratio of loading to Cur amount with the loading efficiencies of 100 to 200% based on the best conditions to achieve the stability of loaded Cur [4]. The optimization method was performed using the numerical analysis method according to desirability functions [24-26]. Three targets of 100, 150, and 200% loadings were selected for the optimization. The optimal factors of Cur/GO and ethanol fraction were then proposed by the optimization software and were examined experimentally. Table 3 represents the optimal conditions with respect to responses and comparison between the experimental and model results.

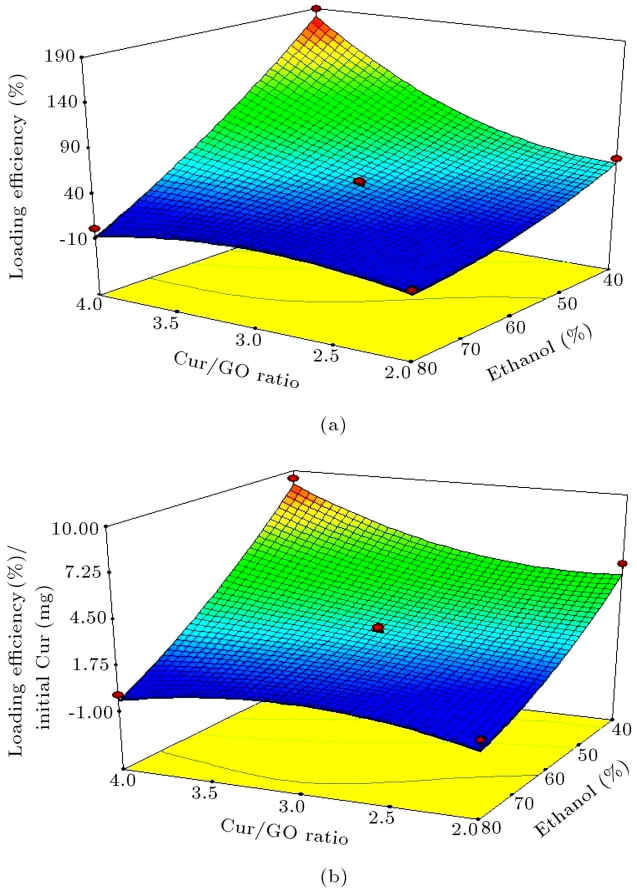

Figure 8. Graphical interpretations: (a) Loading efficiency model, and (b) loading efficiency per weight of initial curcumin ratio.

5.4. Stability study in the optimal condition

After the verification of accuracy of the models with respect to the loading efficiency of Cur onto GO and optimizing the effective factors, the stability of the samples was investigated. For this purpose, Cur was loaded onto GO under optimal conditions, and the identical concentrations for the three proposed samples in phosphate buffer (pH = 7.4) were prepared. After 30 minutes, the sample with efficiency of 180% was precipitated almost completely, and its particles with efficiency of 150% were accumulated slowly and started to precipitate. After 2 hours, samples with efficiencies of 180% and 150% were precipitated completely; however, the sample with efficiency of 100% remained in solution (Figure 9).

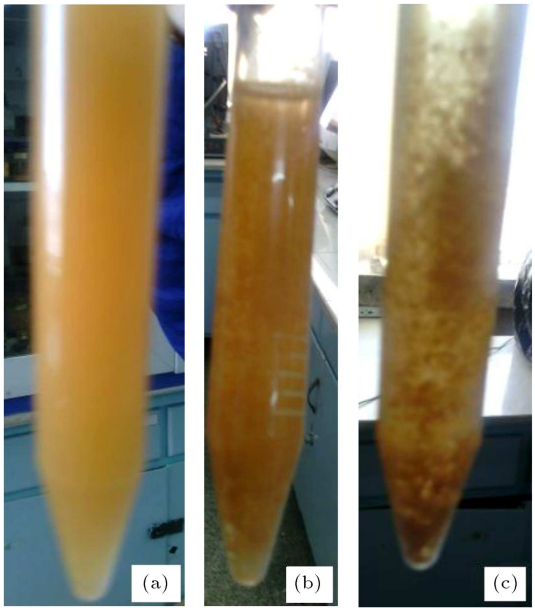

Figure 9. Investigation of GO/Cur loading stability in the optimal condition and loading efficiency of (a) 100%, (b) 150%, and (c) 180%.

Table 3. Comparison between model and experimental results.

Run	Cur/GO	Ethanol (%)	Experimental results		Statistical model		Relative error	
			L (%)	L (%)/Cur (mg)	L (%)	L (%)/Cur (mg)	L (%)	L (%)/Cur (mg)
1	1.66	25.6	112.5	13.554	100	9.212	0.111	0.320
2	2.97	20.2	158.6	10.680	150	10.122	0.054	0.052
3	3.27	21	173.1	10.587	180	11.005	0.040	0.039

L: Loading efficiency; L/Cur: Ratio of loading efficiency to initial Cur.

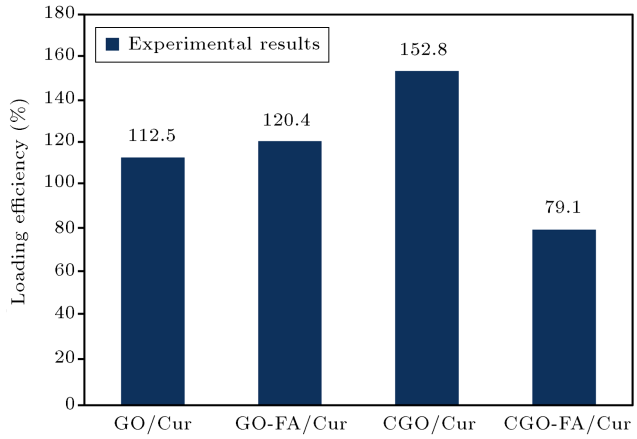

Figure 10. Loading efficiency of curcumin onto carriers in the optimal condition.

Therefore, a comparison of the stability of the three samples showed that the sample with efficiency of 100% was the suitable one because of its higher loading efficiency, lower initial Cur weight, and greater stability. It appears that more Cur loaded onto GO accelerates agglomeration of the particles and reduces the stability of solution.

Cur with solubility of about 6-7 μg/ml in aqueous solutions is almost insoluble in water [27]. After loading Cur onto GO (with the efficiency of 112.5%), its solubility in phosphate buffer increased by almost 1.06 mg/ml.

Following the selection of the optimum point with respect to stability (GO to Cur ratio of 1.66 and ethanol percentage of 25.6 in the solvent), the combinations of GO-FA/Cur, CGO/Cur, and CGO-FA/Cur were also prepared with the optimized GO/Cur and ethanol percentage, and the loading efficiency was calculated. As shown in Figure 10, loading efficiency of Cur on CGO was higher than the other carriers; the reason may be the presence of carboxyl and hydroxyl functional groups in the structure of CA and formation of hydrogen bonds with phenolic groups in Cur. CGO-FA had lower loading efficiency than the other carriers did, because some of the functional groups were occupied by CA and FA, while CGO-FA would be a better carrier because of its greater stability; moreover, it can be used as a targeted delivery agent.

6. Conclusion

The graphene family, especially GO, showed great efficiency to use for loading of hydrophobic and hydrophilic drugs. GO with ligands can be used as nanocarriers for targeted drug delivery. Moreover, properties such as high capacity for drug-loading, biocompatibility, ease of functionolization, and capacity for functionolization with several functional groups created great interest with GO as a nanocarrier. Stable carboxylated GO was produced in physiological solutions by attaching CA to GO through covalent bonds. Moreover, targeted nanocarriers were prepared by attaching FA to GO through covalent bonds. Furthermore, the CGO-FA nanocarrier was also produced by attaching both CA and FA to GO. The anticancer drug Cur was then loaded onto these carriers. Results indicated that loading efficiency improved with increasing the weight ratio of Cur to the carrier and with the reduction of ethanol fraction in the solvent. The efficiency of Cur loading onto CGO is higher than that of GO-FA, and the loading efficiency of Cur onto GO-FA is higher than that of GO. The efficiency of Cur loading onto CGO-FA is the lowest among the others. Despite the lowest loading capacity, this carrier enjoys having a great value due to the presence of two factors making it a stable carrier for targeted drug delivery. The optimal conditions for this carrier were predicted to be as GO/Cur ratio of 1.66 and ethanol percentage of 25.6 in the solvent.

Nomenclature

SEM	Scanning Electron Microscopic
FTIR	Fourier Transform Infrared
Cur	Curcumin
CA	Citric Acid
FA	Folic Acid
GO/Cur	Cur-loading on graphene oxide
CGO/Cur	Cur-loading on CGO
GO-FA/Cur	Cur-loading on GO-FA
CGO	Functionalized graphene oxide by citric acid
GO-FA	Functionalized graphene oxide by folic acid
CGO-FA	Functionalized graphene oxide by citric and folic acids
CGO-FA/Cur	Cur-loading on CGO-FA
GO	Graphene Oxide
GQD	Graphene Quantum Dot
DOX	Doxorubicin
NHS	N-hydroxysuccinimide
RSM	Response Surface Method
ANOVA	Analysis of variance
RCCD	Rotatable Central Composite Design
Cur/GO	Weight ratio of initial Cur to graphene oxide
EDC	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride

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