High-Efficient Loading of Doxorubicin on Graphene Oxide Modifying pH and Temperature

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Graphene Oxide (GO) has been recently introduced as a new efficient drug carrier. GO is biocompatible, has large surface area, and interactive superficial chemical groups. It has been shown that the GO releases loaded doxorubicin (DOX) in acidic pH that is the pH of tumor microenvironment and also cell lysosomes. Therefore, the GO has been presented as a novel carrier for cancer treatment. Because of the 2-D structure of GO, it can carry the drug on its both two-surfaces and edges[1, 2]. Optimizing of effective factors on amount of loaded drug could be result in loading of more drugs on GO.

In this study, the GO was synthetized from graphite using modified hummer's method[3]. After characterization of the synthesized GO using TEM, UV-Vis spectroscopy, Raman spectroscopy and FT-IR, DOX (3.5 mg/ml) was loaded on GO using stirred incubation for 24 hours, followed by several centrifugation-resuspension until complete removement of non adhered DOXs. The loading process was held in different pHs (5.4, 7.4, 9.4) and temperatures (24, 37, 42° C) and the amount of loaded DOX on GO was measured using UV-Vis spectroscopy. To obtain the optimized loading amount of DOX on GO, we use CCD and RSM mathematical model using three-level two-factor fractional factorial CCD, based on experimental results (pH, 5.4, 7.4 and 9.4, Temperature, 24, 37, 42° C) of loaded DOX on GO. The release of loaded DOX in various pH conditions was measured in neutral (physiological plasma) and acidic (cancer celllysosomal) pHs using dialysis method and UV-Vis Spectroscopy.

Our experimental results showed that DOX would be more loaded in basic pH. This could be due to changes in hydrogen-bond interaction of hydroxylic and carboxylic groups of GO surface with DOX. The π-π stacking interactions of DOX and GO may also be modified in basic pHs leading to more interaction between DOX and GO. Just as it seemed, the loading of DOX on GO was more in body temperature (37° C), and it is diminished in higher or lower temperatures than 37 ° C. This result could be explained by less energy to form bonds in lower temperatures and more dissociative energy in higher temperatures that let not to establish bonds. The quadratic polynomial model to identify the optimized pH and temperature for the most loaded amount of DOX on GO revealed that the amount of loaded DOX on GO is related to the first- and second-order terms of pH and the second order of temperature ($P>0.01$). Therefore, the proposed model is:

$R1 = 177.41 + 45.33A - 26.45A^2 - 11.95B^2$, where R1, A and B were the loaded amount of DOX, pH, and temperature, respectively. A positive coefficient in the equation represents a synergistic effect, while the negative sign indicates an antagonistic effect. Finally, the optimized pH and temperature for highest loading of DOX on GO are 9.12 and 37° C, respectively. The cumulative release of DOX loaded on GO in obtained optimized pH (9.12) and temperature (37° C)after 72 hours was 61.7% in acidic pH and 41.2% in neutral pH. As it is obvious, the realse of loaded DOX in acidic pH was more than neutral pH.

In conclusion, we optimized the pH and temperature for loading of the most applicabale amount of DOX on GO with acceptable release in acidic pH that could be used for novel approchs of cancer drug delivery.

References

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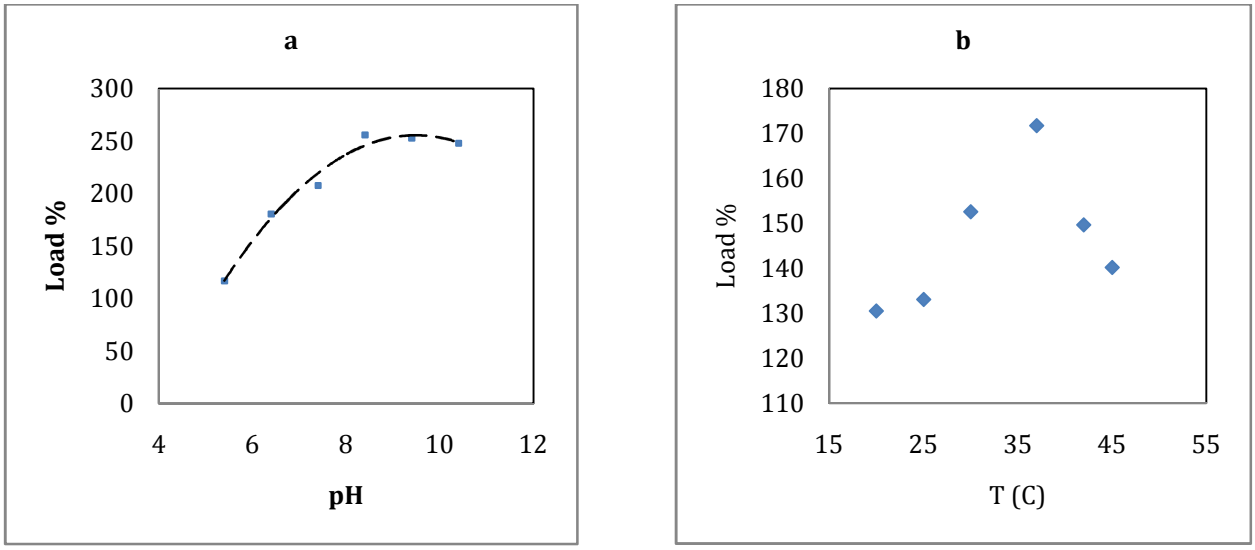

Figure 1: The effects of (a) pH and (b) temperature on loading of DOX on GO. In basic pH and body temperature, DOX has the highest loadiing on GO.

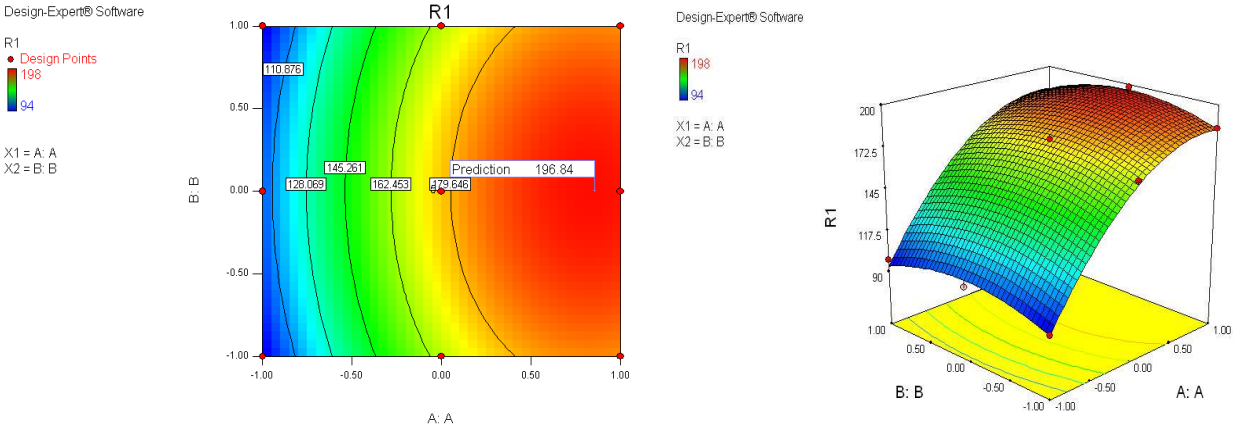

Figure 2: Graphs of mathematical modeling to obtain the optimized values of pH and temperature for highest loading of DOX on GO. Based on this model the optimized pH and temperature for highest loading of DOX on GO is 9.12 and 37° C, respectively.

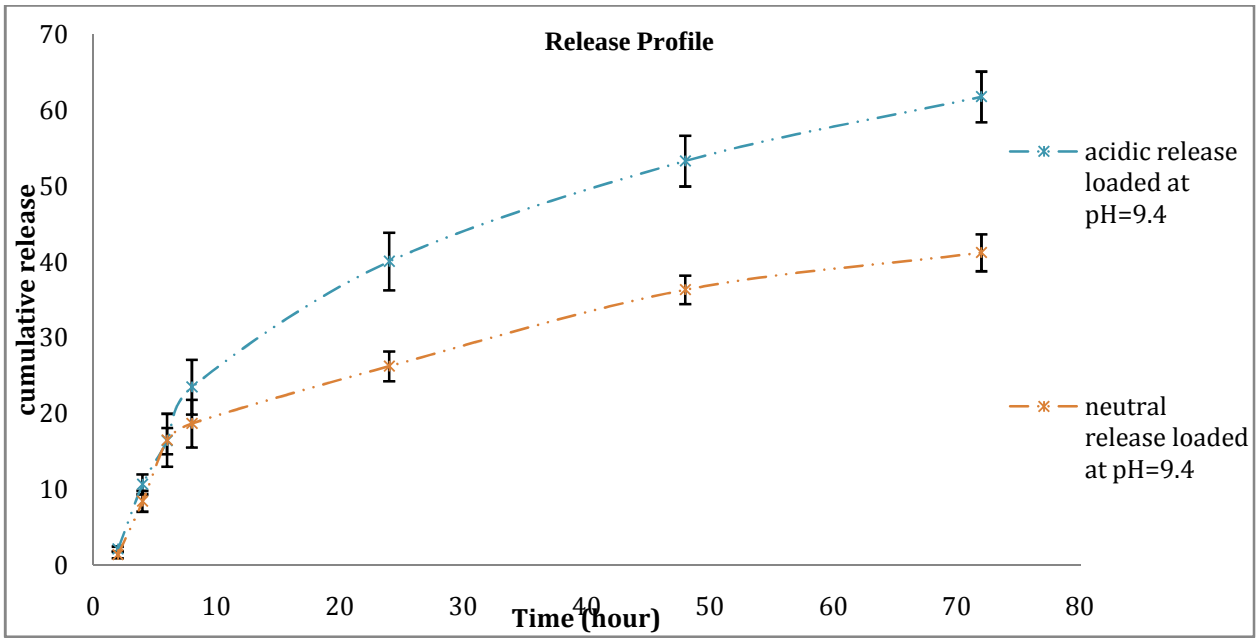

Figure 3: Cumulative release of DOX loaded in optmized pH (9.12) and temperature (37° C). cumulative release was 61.7% in acidic pH and 41.2% in neutral pH after 72 hours.