The Testing Method of Graphene Oxide (GO) and NAA Ramification

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Abstract. Organic/inorganic supramolecular system has been obtained by graphene oxide grafting with α-cyclodextrins (α-CDs) and NAA ramification. Fourier transform infrared spectra (FTIR), thermogravimetric analysis (TGA), X-ray diffraction (XRD), differential scanning calorimetry (DSC), and X-ray photoelectron spectroscopy (XPS) were employed to identify the characters and morphology of the supramolecular structure. The features might make our supramolecular hybrid hydrogel a pretty candidate in a drug delivery system.

Keywords: Graphene, NAA branching, drug delivery system.

1. Introduction

In recent years, research work has been devoted to the preparation of graphene functional composites by surface modification of graphene oxide. For example, Chen et al. [1] reported the application of phenyl modified graphene oxide instead of graphene in the study of organic photovoltaic devices of electron acceptors; Dai et al. [2] prepared PEG modified graphene oxide derivatives, which can introduce water-insoluble anticancer drug molecules into the water phase. α - naphthylacetic acid (α - NAA) is a kind of plant growth regulator, which can increase the production of vegetables, prevent plants from falling flowers and fruits and induce flowering [3]. In this paper, the organic - NAA / - CD hybrid gel was prepared in aqueous solution by using alpha -NAA to bind to the cavity of beta -CD through supramolecular force. This material has potential application prospects in the field of biomedicine.

2. Experimental part

2.1. Main raw materials and instruments equipment

The main raw materials used in this experiment are: natural scale graphite, Qingdao Guangli graphite Co., Ltd.; β - cyclodextrin, Shanghai Boao Biotechnology Co., Ltd.; p-methylbenzenesulfonyl chloride, acetonitrile, N-hydroxysuccinimide, 1-ethyl-3 - (3-dimethylaninopropyl) carbodiimide hydrochloride, 4-dimethylaminopyridine, Dicyclohexylcarbodiimide. Main instruments and equipment: vacuum drying oven, ultrasonic cleaner, X-ray photoelectron spectrometer, X-ray diffractometer, thermogravimetry, etc.

A subsection
2.2. Preparation of graphene oxide aqueous solution by Hummer’s method
Take a proper amount of natural flake graphite and NaNO3 in a three port flask, add concentrated sulfuric acid under the ice water bath and low-speed stirring, and stir for 30 min; add a proper amount of KMnO4, stir for 24 h, add dilute H2SO4, and continue to stir for 2 h; after the reaction, add H2O2 drop by drop until no gas is generated; after that, take the precipitated black solid by high-speed centrifugation, which is graphene oxide.

2.3. Synthesis of EDA monosubstituted β – CD
Add CH3CN solution of p-TsCl to β - CD aqueous solution drop by drop; then, react at 25 ℃ for 2 h, neutralize with dilute HCl to pH = 6 ~ 7, filter and precipitate, recrystallize with deionized water and dry to obtain β - cd-6-ots. Dissolve it in EDA and react for 6h at 75 ℃; after cooling to room temperature, drop it into C3H6O to precipitate, filter by suction, dissolve the filter cake in the aqueous solution of methanol, then precipitate it in acetone again, filter by suction and dry it to obtain the white solid as EDA - β - CD.

2.4. Synthesis of graphene oxide modified by β – CD
Take graphene oxide and disperse it in PBS solution, then add EDA - β - CD, EDC • HCl and NHS into the system, after 24 hours at room temperature, dialysis and drying, the black solid is graphene oxide modified by β - CD (go-cd)

2.5. Synthesis of peg-2000 (n aa-peg) with NAA termination
Take peg-2000 and put it into a round bottom flask, add dichloroethane, stir it with magnetic force to make peg-2000 completely dissolved; after that, respectively put α - NAA, DMAP and DCC into the flask; after reaction at room temperature for 24 hours, filter it and wash the filter cake with dichloroethane for several times; spin the filtrate, slowly drop it into excessive ether to precipitate and filter, and the light yellow solid is NAA peg.

2.6. Construction of supramolecular assembly (go-cd / n aa-peg) of go-cd and n aa-peg
Take the go-cd bottle placed in the sample bottle, add appropriate amount of deionized water, disperse by ultrasonic, add NAA-PEG to the sample bottle, stir 24 h at room temperature, and form a black gel at the later stage of reaction.

The construction process is as follows:

Fig 1. Schematic figure of the construction of go-cd/PEG-NAA
3. Results and Discussion

3.1. XPS analysis of graphene oxide and go-cd powder

As can be seen from the above figure, the surface of graphene oxide lamellae contains a large number of oxygen-containing groups, such as hydroxyl, carboxyl and carbonyl groups; in addition, through XPS analysis, the composition proportion of C, N and O atoms in graphene oxide can be obtained, as shown in Table 1; comparing the composition proportion of C and O atoms in graphite helps us to distinguish the original graphite from graphene oxide materials.

In general, natural flake graphite contains about four Wt% of oxygen is due to the oxidation of graphite by O2 in the air [5]. However, the content of oxygen in graphene oxide is much higher than that of natural flake graphite, which is about thirty-five point six two Wt%, but less than 50 wt% (oxygen content of carbon monoxide). This data shows that the oxidation degree is high, but it has not completely decomposed into carbon monoxide gas.

![Fig 2. XPS patterns of the graphene oxide powders (a) O 1s, (b) C 1s, (c) N 1s, (d) wide scan and the XPS patterns of the go-cd powders (e) O 1s, (f) C 1s, (g) N 1s, (h) wide scan](image-url)
From the results of XPS analysis of go-cd and graphene oxide powder, it can be found that the content of N atom has nearly doubled, which is caused by ethylenediamine grafted on CD. In addition, the existence of CD changes the ratio of original C and O of graphene oxide, and the content of C and O increases or decreases correspondingly, which indicates the successful preparation of go-cd.

**Tab1.** Atomic composition of go-cd and graphene oxide testified by XPS

| sample  | Atomic composition ratio (%) |
|---------|------------------------------|
|         | C    | N    | O    |
| GO      | 63.80| 0.58 | 35.62|
| go-cd   | 67.98| 0.98 | 31.03|

3.2. 1H NMR characterization of EDA - β - CD

![1H NMR spectrum of EDA-β-CD](image)

**Fig 3.** 1H NMR spectra of EDA-β-CD solved in D$_2$O

Fig. 3 is the EDA - β - CD NMR spectrum. The peak of benzene ring hydrogen at the chemical shift of 7.75-7.43 ppm disappears, and the peak of methyl hydrogen at 2.43 ppm also disappears, which indicates that p-toluenesulfonyl has been replaced; the peak of chemical shift at 2.83-2.78 ppm corresponds to the peak of a and B near the amino group. In conclusion, the target product EDA - β - CD has been successfully prepared.
3.3. *FTIR analysis of go-cd*

Fig. 4. FTIR spectra of go-cd

Fig. 4 is the infrared spectrum of go-cd. The absorption peak at wave number 3443 cm⁻¹ is O-H, and the stretching vibration peak at N-H. The peaks at wave number 2920 cm⁻¹ and 2850 cm⁻¹ respectively correspond to the methylene symmetric vibration absorption peak and the asymmetric vibration absorption peak. The existence of these two peaks is caused by the introduction of CD; 1553 The wave peak at cm⁻¹ is the bending vibration of the secondary amide bond. On the other hand, it shows that CD is modified to graphene oxide by amide bond.

3.4. *TGA analysis of go-cd*

Fig 5. TGA spectra of go-cd
Fig 5. is the TGA diagram of go-cd, the weight loss of go-cd between 250 °C and 400 °C, which is mainly caused by the thermal decomposition of β - CD; the 9.55 wt% weight loss of the first stage and the 15.45 wt% weight loss of the second stage correspond to the decomposition of the oxygen-containing group and β - CD of graphene oxide in go-cd respectively; therefore, we successfully synthesized the graphene oxide modified by β - CD through amidation reaction.

3.5. The LPSA Test

As shown in the figure above, the particle size of graphene oxide, go-cd and go-cd-PEGNAA in aqueous solution is analyzed and tested by laser particle size analyzer. It can be seen from the figure that the particle size of graphene oxide, go-cd and go-cd-PEGNAA in turn increases: 35% of graphene oxide particles are in the range of 600-700nm, 40% of graphene oxide particles are in the range of 600-700nm, 50% of go-cd-PEGNAA particles are in the range of 600-700nm In the range of nm, it can be seen that the particle size of go-cd-PEGNAA is not large compared with graphene oxide and go-cd, which may be caused by the low density of the network connection point in the system, the low concentration of go-cd-PEGNAA in the dispersion system, and the inclusion of CD and NAA is easy to fall off under ultrasound. This result proves the successful construction of graphene oxide based supramolecular assembly.

4. Conclusion

In this paper, (1) graphene oxide was prepared by Hummer's method. By XPS analysis, the ratio of oxygen atom in graphene oxide was 35.62%, and β - CD was grafted on graphene oxide to prepare go-cd. By TGA test, the ratio of β - CD to go-cd was 15.45 wt%; (2) NAA was grafted on both ends of peg-2000 by DCC shrinkage esterification NMR analysis showed that the grafting was complete; (3) the self-assembly of go-cd and peg-naa was completed in aqueous solution by using the supramolecular inclusion of NAA and β - CD. LPSA analysis showed that the particle size of the assembled go-cd-PEGNAA was significantly larger than that of graphene oxide and go-cd.

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