

COPPER INCORPORATED NANO-GRAPHENE-NHC COMPLEX DESIGNED FOR ANTIBACTERIAL ACTIVITY

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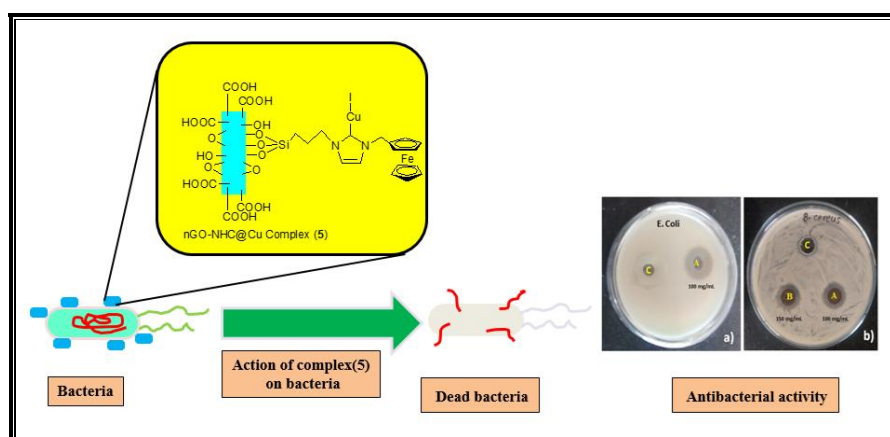
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Abstract:

An efficient Copper incorporated nano-graphene-NHC complex acronymed as nGO-NHC@Cu complex (5) has been synthesized by covalent grafting of ferrocenyl ionic liquid in the matrix of nano-graphene followed by metallation with copper (I) iodide. The nGO-NHC@Cu complex (5) was successfully characterized by Fourier transform infrared (FT-IR), Thermogravimetric analysis (TGA), X-ray Diffractometer (XRD), energy dispersive X-ray analysis (EDX), Brunauer-Emmett-Teller (BET) and Transmission electron microscopy (TEM) analysis. nGO-NHC@Cu complex (5) displayed in vitro antibacterial activity against gram negative and gram-positive bacterial species such as *Escherichia coli* (NCIM-2832) and *Bacillus cereus* (NCIM-2703).

Keywords: Nano-Graphene, NHC, Copper Iodide, Antibacterial Activity.

Graphical abstract:



1 Introduction

From the last ten years, *N*-heterocyclic carbenes (NHCs) have been attracted to researchers as powerful tools as ligand in organic chemistry and biological applications (1). In addition, astonishing properties of NHCs such as good σ -donor ability offer advantages of high stability in physiological media and excellent pharmacokinetic properties (2). Several recent studies focusing on antibacterial activity of various NHC-metal complexes have highlighted their promising potential as novel therapeutic agents (3) However, copper NHC have received special attention in antibacterial activity for their great potential (4) Despite of tremendous research in this field there is still scope to develop new antibacterial agents.

Nanomaterials play a pivotal role in biological applications as they enhance the antibacterial properties via new therapeutic agents. Recently, graphene oxide (GO) has emerged as one of the most promising nanomaterials. GO has been widely applied in diverse areas including antibacterial activity (5). Chemical properties of GO can be easily tailored by facile introduction of desired functional groups. In addition, large surface area and chemical stability of GO facilitate high loading of active sites. These attributes have made GO as a sustainable and versatile support for the development of tailor-made therapeutic agents (6). However, despite tremendous advances, there is considerable scope for the further development especially using nanomaterial.

Based on aforementioned discussion and in continuation of our studies related to graphene and medical applications (7,8), we report herein preparation of copper incorporated nano-graphene-NHC complex and its application in the antibacterial activities.

2 Experimental: Material and Methods

2.1 Preparation of nano-GO [nGO] (1)

A mixture of graphite oxide (1.0 g) and distilled water (250 mL) was sonicated for 30 min. Subsequently, 50 % hydrazine hydrate (5 mL) was added slowly. The resulting mixture was refluxed in an oil bath. After 4 h, insoluble product was separated by centrifugation, washed with water (6 × 20 mL) and dried under vacuum at 50 °C to afford nGO (1).

2.2 Preparation of chloropropyl modified nGO (2)

A mixture of **1** (8.0 g) and (3-chloropropyl) triethoxysilane (10.8 mL, 45 mmol) in xylene (50 mL) was refluxed in an oil bath. After 24 h, the mixture was cooled and the residue was separated by centrifugation washed with THF (3 × 5 mL) and dried under vacuum at room temperature to afford chloropropyl modified nGO (2).

2.3 Preparation of [nGO-FemImi]Cl (4)

A mixture of **2** (8.0 g) and 1-*N*-ferrocenylmethyl imidazole (**3**) (3g, 11 mmol) in DMF (25 mL) was heated at 80 °C in an oil bath. After 72 h, the solid was separated by centrifugation, washed with DMF (3 × 50 mL), MeOH (3 × 50 mL) and CH₂Cl₂ (3 × 50 mL) and dried under vacuum at 50 °C for 24 h to afford [nGO-FemImi]Cl (4).

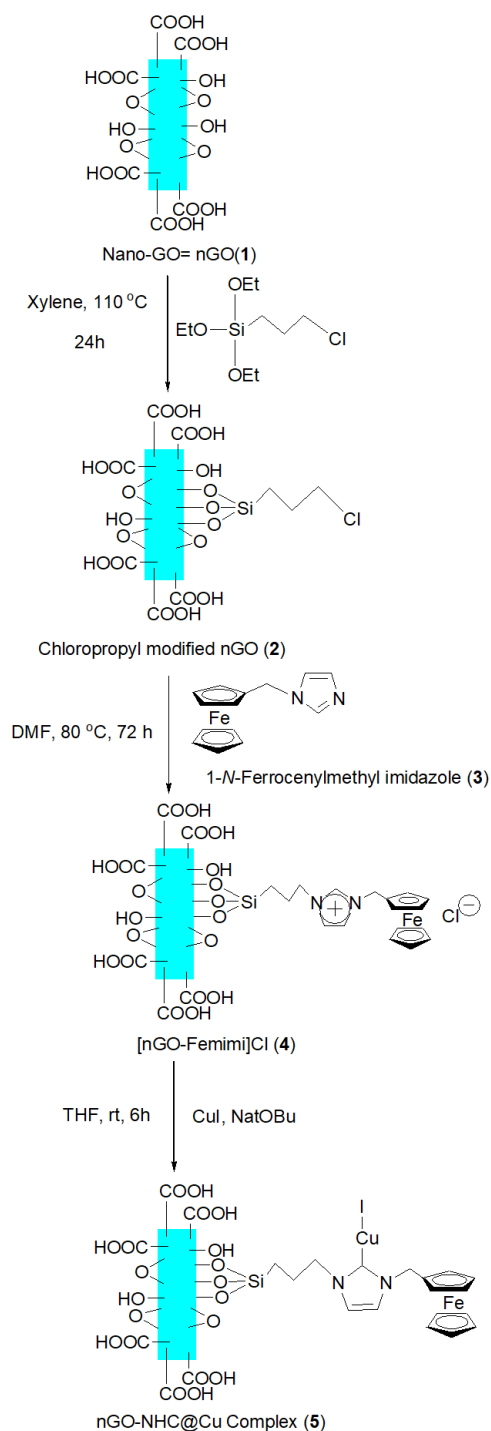
2.5 Preparation of nGO-NHC@Cu complex (5)

A mixture of **4** (9.0 g), CuI (1.9 g, 10 mmol) and NaOtBu (0.096 g, 10 mmol) in THF (50 mL) was stirred at room temperature under nitrogen for 6 h. Afterwards, the mixture was separated by centrifugation, washed with THF (3 × 20 mL) and dried under vacuum for 24 h to afford nGO-NHC@Cu complex (5).

3. Results and Discussion

The preparation of the Copper incorporated nano-graphene-NHC complex is outlined in Scheme 1. Initially, nGO (1) prepared by modified Hummers and Offeman method. (9,10) The **1** was subsequently treated with (3-

chloropropyl)triethoxysilane to form chloropropyl modified nGO (2). The synthetically fertile chloro group in 2 allowed installation of azolium group in the nano-GO matrix through quaternization with 1-*N*-ferrocenylmethyl imidazole (3) to yield heterogeneous salt acronymed as [nGO-FemImi]Cl (4) that served as precursor for preparation of NHC-Cu complex. Finally, the complexation of 4 with CuI yielded the desired Copper incorporated nano-graphene-NHC complex acronymed as nGO-NHC@Cu complex (5).



Scheme 1: Preparation of nGO-NHC@Cu complex (5)

The prepared complex **5** has been characterized by various techniques summarized as below.

3.1 FT-IR analysis: The FT-IR spectrum of **5** displayed characteristic peaks at 3437 cm^{-1} (O-H stretching), 2885 cm^{-1} (C-H stretching), 995 cm^{-1} (Si-O stretching), 682 cm^{-1} (C-Cl stretching). The appearance of peaks at 470 cm^{-1} (Fe-Cp stretching band), 1385, 1463 and 1575 cm^{-1} (ring stretching modes of imidazolium ring), 2921 and 2852 cm^{-1} (C-H stretching of Cp rings), 1642 cm^{-1} (C=C stretching), 1575 cm^{-1} (N-C-N stretching), 3410 cm^{-1} (O-H stretching) demonstrated formation of **5**(11).

3.2 TGA analysis: The thermal stability profile of nGO-NHC@Cu complex (**5**) was assessed by TGA analysis over the temperature range of 25-1000 $^{\circ}\text{C}$ at heating rate of 10 $^{\circ}\text{C}/\text{min}$. The initial weight loss of 5.3 % up to 185 $^{\circ}\text{C}$ is due to desorption of physically adsorbed water. The second key weight loss of 10.5 % up to 308 $^{\circ}\text{C}$ is attributed to the weight loss of pendant ferrocenyl group and surface bound organic scaffolds. The third weight loss of 13.8 % corresponds to the decomposition of the graphene by oxidative mode up to the 380.20 $^{\circ}\text{C}$. The two combined weight losses of 30.6 % and 18.1 % are assigned to complete combustion of GO and carbon skeleton. The observations are in good agreement with TGA profile of nGO reported in the literature (12).

3.3 XRD analysis : The X-ray diffraction (XRD) pattern of nGO-NHC@Cu complex (**5**) displays two noticeable diffraction peaks at 26.0 $^{\circ}$ and 43.3 $^{\circ}$, corresponding to the (002) and (101) reflections of graphitized carbon, respectively suggesting structural integrity of support(13).

3.4 EDX analysis: The amount of Cu in of nGO-NHC@Cu complex (**5**) was quantified by using energy dispersive X-ray (EDX) analysis. The analysis revealed presence of 0.26 mmol of Cu per gm of **5**.

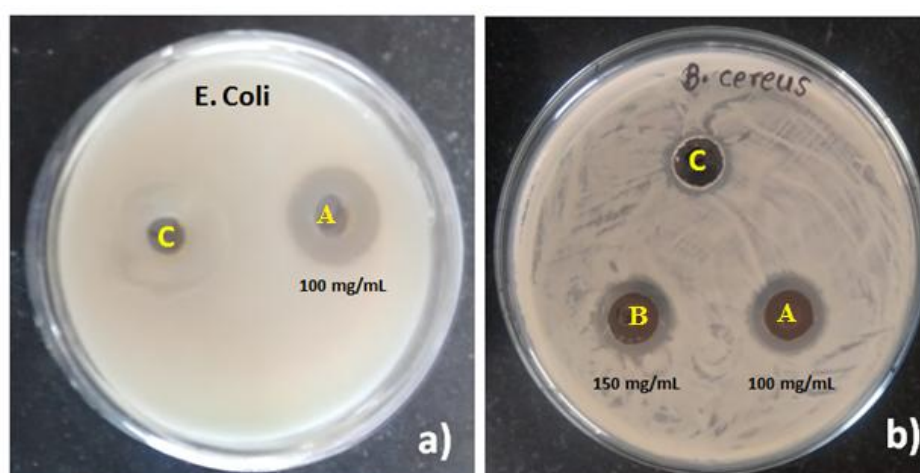
3.5 BET analysis: Brunauer-Emmett-Teller (BET) surface area analysis was performed to evaluate the textural properties of of nGO-NHC@Cu complex (**5**). The **5** showed BET surface area of 20.23 m^2/g with the average pore diameter of 43 A° . The surface area, pore volume and pore size of **5** was significantly smaller as compared to pristine graphene oxide (77 m^2/g , 23 A°) indicating loss of micropores due to substantial grafting of functional groups on the surface of graphene.

3.6 TEM analysis: Transmission electron microscopy (TEM) was employed to warrant morphological aspects of of nGO-NHC@Cu complex (**5**). The TEM micrographs displayed crumpling features with twisted nanosheets in disorderd phase. The fold edges by virtue of sp^3 carbon in nanosheets of nGO carry immobilized NHC-Cu complex on both sides facilitating easy access of catalytic sites to the reactants. (14).

3.7 Antibacterial activity: we diverted our attention toward evaluating antibacterial activity of nGO-NHC@Cu complex (**5**). The *in vitro* antibacterial activity of nGO-NHC@Cu complex (**5**) was evaluated against Gram negative *Escherichia coli* (NCIM-2832) and Gram positive *Bacillus cereus* (NCIM-2703) pathogenic bacterial strains (Table 1). The studies reveals that **5** displayed strong antibacterial activity against *Escherichia coli* (NCIM-2832) with maximum zone of clearance of 17 mm for 100 mg of **5** (Fig. 1a) while moderate antibacterial activity against *Bacillus cereus* (NCIM-2703) with minimum zone of clearance of 12 mm and 14 mm for 100 mg and 150 mg of **5** respectively (Fig. 1b). The moderate antibacterial activity of **5** against *Bacillus cereus* (NCIM-2703) is attributed to fact that cell wall of Gram positive strain is wider than Gram negative strain. The antibacterial studies demonstrated that **5** shows significant antibacterial activity against both Gram-negative *Escherichia coli* (NCIM-2832) and Gram-positive *Bacillus cereus* (NCIM-2703) bacterial strains.

Table 1: Antibacterial activity of of nGO-NHC@Cu complex (5) against *Escherichia coli* (NCIM-2832) and *Bacillus cereus* (NCIM-2703)

Sr. No	Concentration of nGO-NHC@Cu complex (5) (mg/mL)	Well in plate	Zone of inhibition For <i>Escherichia coli</i> (NCIM-2832) (mm)	Zone of inhibition For <i>Bacillus cereus</i> (NCIM-2703) (mm)
1	100	A	17	12
2	150	B	-	14
3	0	C	Absent	Absent

**Figure 1: Zone of inhibition due to nGO-NHC@Cu complex (5) around the well in plate inoculated with (a) *Escherichia coli* (NCIM-2832); (b) *Bacillus cereus* (NCIM-2703)****Conclusion**

In conclusion, we have successfully prepared Copper incorporated nano-graphene-NHC complex acronymed as [nGO-NHC@Cu complex (5)] and characterized by FT-IR, TGA, XRD, EDX, BET and TEM analysis. The 5 exhibits significant *in vitro* antibacterial activity against pathogenic bacterial strains such as Gram negative *Escherichia coli* (NCIM-2832) and Gram positive *Bacillus cereus* (NCIM-2703). These findings suggested that [nGO-NHC@Cu complex (5)] can be further explored for potential applications in the field of various biomedical research.

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