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SYNTHESIS OF GADOLINIUM GRAPHITIC-CARBON NITRIDE (Gd/g-C_3N_4) COMPOSITE FOR THEIR BIOLOGICAL APPLICATIONS

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Abstract

In this study, we reported on the possibility of the new material gadolinium-doped carbon nitride (Gd/g-C_3N_4) composite and last documented checks for its antimicrobial properties, antioxidant, and biocompatibility. The Gd/g-C_3N_4 composite was prepared and evaluated for biotoxicity and antioxidant activity, bacterial biofilm inhibition in *"Escherichia coli" and "Staphylococcus aureus".* Cytotoxicity profiles were further analyzed with the help of hemolysis assay and as per the results obtained, Gd/g-C_3N_4 exerted mild eryptosis, 10.05% while g-C_3N_4-DMF, 4.78%. The anti-radical activities were predicted using DPPH assay; g-C_3N_4-DMF showed a higher percentage radical scavenging ability of 48.09 % than Gd/g-C_3N_4 of 36.27 %. For antibacterial testing, the potential to inhibit the "E. coli" and "S. aureus" bio movie formation was analyzed, and it could be seen that the developed Gd/g-C_3N_4 significantly performed slightly better compared to the g-C_3N_4-DMF. These results show that the Gd/g-C_3N_4 composite possesses relatively satisfactory biological properties, but the

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biocompatibility and the antibacterial performance of the material should be enhanced when applied to disinfection and antifouling coatings.

Keywords: Gadolinium (Gd), Carbon nitride(g-C_3N_4) Composite, synthesis, antimicrobial, antioxidant, biocompatibility, assessment, material science, biomedical applications.

1. Introduction

Self-assembling materials have thus found plenty of focus in the past few years due to the rising utility of micro-nanostructured multifunctional materials for Biomedical and environmental uses. Of the above materials, composites with Gd have received even more attention due to their physicochemical and biological properties. [1]Gadolinium (Gd), a relatively more tractable member of the rare-earth family, has found numerous applications in MRI contrast agents, catalysis, and material science due to its high magnetic moment, thermal stability, and fascinating coordination chemistry. In the biomedical application of biomedical composite materials science this lead, gadolinium with added composites can be filled to enhance the antimicrobial, antioxidant, and biocompatibility characteristics which could act as good candidate for various therapeutic and diagnostic applications.[2]

The role of two-dimensional carbon nitride (g-C_3N_4) is functional and potentially photocatalytic, electronic and structural. This material has high thermal and chemical stability and its desirable bandgap is also tunable according to need.[3] Moreover, successful synthesis of stable composites with metals and any other groups. The use of gadolinium with carbon nitride will therefore improve the characteristics of both the materials physiochemically and biologically as the interaction notably forms a mainly synergistic material.[4] Such composites have also all possibilities for application in biomedical fields for protection against microbial adhesion, for drug delivery systems or as artificial tissues for support.[5]

Among the major problems faced by today's health care system, bacterial infection and free radical mediated stress constitute two major problems that cause conditions such as, impaired wound healing, bacterial biofilm, and chronic diseases. [6]The ongoing increase in the rate of antibiotic resistance Spread only emphasizes the need for other ways to fight antimicrobial infections. These materials have done away with normal antibiotics and are a viable solution for guarding famous materials against bacteria. [7]Moreover, the oxidative stress appears to be responsible for the etiopathogenesis of many diseases including cancer,

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diseases of the central nervous system and cardiovascular disease when the rate of ROS production outstrips the body's antioxidant defenses. They key idea that allows one to overcome these challenges is to produce materials that combine both antimicrobial and antioxidant characteristics.[8]

Surprisingly, biocompatibility appears to be a big consideration for any material intended for biomedical application. In engaging the material with biological systems, it cannot elicit adverse response like inflammation or cytotoxicity. Therefore, evaluation of compatibility of newly developed material is of paramount importance before their application in clinical medicine.[9]

In this research we prepared and characterized a NEW gadolinium carbon nitride (Gd-C_3N_4) and also evaluate its antimicrobial activity, antioxidant activity and biocompatibility. The synthesis process is, as a matter of fact, very much simplified and relatively very flexible for further scaling and the enhancement of the functionality of the carbon nitride matrix relies on the doping of the gadolinium ions. [10]The general efficiency of the composite in terms of virucidal properties is tested with bacterial and fungal test organisms, which gives consideration on how the composite is applied as a broad-spectrum antimicrobial agent.[11] To determine the antioxidant efficacy, the abilities of antioxidants to scavenge free radicals and other oxidative species in the material are investigated. Last, it is in vitro biocompatibility is tested to verify that it is suitable for use in biomedicine.[12]

The current work also illustrates the potential and importance of the Gd-C_3N_4 composite in applying point material science to biomedical usage. Consequently, the results of this study can act as foundational knowledge for developing new networked nanomaterials for healthcare and environmental applications.[13]

2. Experimental Effort

2.1 Synthesis of Gadolinium-Carbon Nitride Composite

2.1.i Preparation of Carbon Nitride (C_3N_4):

The graphitic carbon nitride (g-C_3N_4) has been prepared through the thermal polymerization of nitrogen-containing compounds including melamine, dicyandiamide, urea, and cyanamide. He then heats the weighed precursor in a crucible under nitrogen or argon atmosphere in a muffle furnace at a temperature range of 500°C – 600°C, 2-4 hours holding

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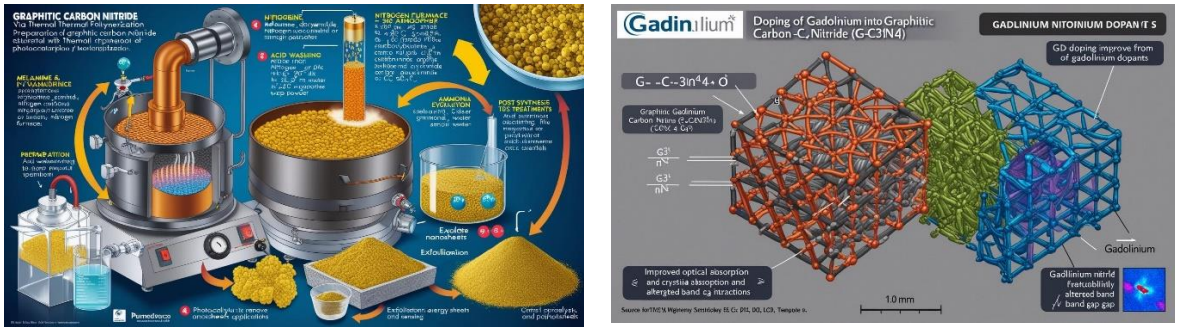
time, and a ramp rate of approximately 5°C / minute. [14]Copper-role has an urge to make this thermal treatment as this leads to the formation of gaseous by-products like ammonia and water. This yields a yellow or light-brown material that is graphitic carbon nitride. Other treatments treated in the post-synthesis are acid washing to remove impurities and exfoliate the nanosheets. Doping with metals or nonmetals can also vary the electronic and optical characteristics of the material. The final product material has utility in photocatalysis, energy storage, sensing, and other technologies.[14]

2.1.ii Doping of Gadolinium into g-C_3N_4:

The incorporation of gadolinium into graphitic carbon nitride (g-C_3N_4) brings about improvement in its photocatalytic, electronic, and optical characteristics. Gd doping can be done before the synthesis of C_3N_4 or may be done after the synthesis of C_3N_4. In the in-situ method, Gd ions are deposited in the C_3N_4 matrix which is prepared by combining nitrogen-containing precursors such as melamine or urea and then heating the mixture in a nitrogen or argon atmosphere between 500–600°C. [15]Otherwise, post-synthesis doping can be done in a manner with dissolving primary g-C_3N_4 synthesized in the Gd precursor solution followed by the calcination process. Doping is further confirmed by the XRD, FTIR, and UV-visible and photoluminescence spectroscopy evidence of improved optical absorption, decrease in bandgap, and better charge dynamics required for photocatalytic applications such as hydrogen production and environmental remediation.[15]



(a) (b)

Figure:2.1 (a) Preparation of Carbon Nitride (C_3N_4) (b) Doping of Gadolinium into g-C_3N_4

2.2 Antimicrobial Activity Assays

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Herein the MAP of Gd-doped graphitic carbon nitride (Gd/g-C_3N_4) is tested through the well-diffusion, disc-diffusion, and shake flask methods against bacterial and fungal strains. The effectiveness was proved by the ability to form inhibition zones and increase the OD in liquid media. The improvement of the antimicrobial performance of Gd/g-C_3N_4 is attributed to the production of radical oxygen species and its effect on microbial enzymes. It is therefore useful in biomedical and communal technology areas.[16]

2.2.1 Well-Diffusion Assay:

The growth inhibition ratio is evaluated in the context of the so-called well-diffusion assay for bacterial and fungal organisms including *"Escherichia coli" and "Staphylococcus aureus".* Inhibition zones around the wells containing Gd/g-C_3N_4 material prevent microbial growth in the case of samples/ultrasound. It is thereby used to describe Gd/g-C_3N_4 which is a photocatalytic material capable of generating reactive oxygen species and at the same time disrupting microorganisms' cell structures hence having enormous utility in medicine/health and industrial sectors.[17]

2.2.2 Disc-Diffusion Assay:

A disc-diffusion assay determines the antibacterial and antifungal efficacy of Gd-doped carbon nitride (Gd/g-C_3N_4). Coated discs include sterilized discs containing Gd/g-C_3N_4 which are placed on agar containing microbial cultures of *"Escherichia coli" and "Staphylococcus aureus".* Inhibition zones indicate that the material possesses antimicrobial properties because of the ability to produce ROS, and to impair the functions of microbial cells; therefore Gd/g-C_3N_4 can be employed in medical and investigative practice.[18]

2.2.3 Shake Flask Method:

The shake Flask Method is used in the determination of antibacterial efficacy from Gd-doped carbon nitride (Gd/g-C_3N_4) using nutrient broth with varied concentrations ranging between 50mu and 400 mu. Antimicrobial growth inhibitions are determined when the amount of OD changes is being measured. By so doing, it is seen that Gd/g-C_3N_4 has a strong positive correlation with concentration and this concentration dependency makes it beneficial in biomedical and environmental applications. [19]

$$\%\text{Growth Reduction} = \frac{\text{OD}_{\text{control}} - \text{OD}_{\text{treated}}}{\text{OD}_{\text{control}}} \times 100$$

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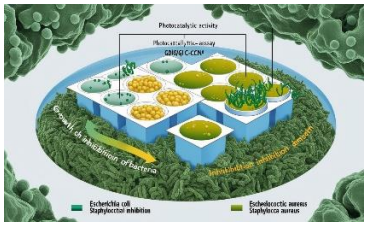

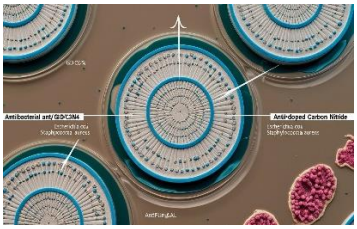

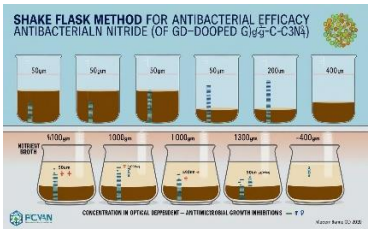

(a) (b) (c)

Figure:2.2 (a) Well-Diffusion Assay (b) Disc-Diffusion Assay (c) Shake Flask Method

2.3 Antioxidant Activity via DPPH Assay

The antioxidant activity of the Gd-doped carbon nitride (Gd/g-C_3N_4) photocatalyst is determined using DPPH free radical solution through interaction with the photocatalyst solution in different concentrations of Gd/g-C_3N_4. The degradation of the purple colours to yellow is quantified using UV-Vis spectrophotometry at 517 nm to prognosticate the scavenging potential of Gd/g-C_3N_4. Its antioxidant capability is accordingly significant since the EC50 value expresses the concentration capable of reducing the radical DPPH by 50%. This property attributed to the electronic effect of gadolinium doping makes Gd/g-C_3N_4 a potential candidate for the food packaging, cosmetics, and biomedical industries.[20]

$$\%\text{Radical Scavenging} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

2.4 Biocompatibility Assay of Gd-Doped Carbon Nitride (Gd/g-C_3N_4)

The cytotoxicity of Gd/g-C_3N_4 of the samples is determined in vitro cytotoxicity and cell compatibility biocompatibility assay through a series of lab analyses. In this, primary cultures of human cells like fibroblasts or epithelial cells are cultured multi-parametrically, and treated with various concentrations of Gd/g-C_3N_4. Quantitative cell responses will be in terms of cytotoxicity using MTT or Live/Dead staining assays. Cell morphology, growth rates, and apoptotic activity are also documented by microscopy analysis. Taken together these results set the safe concentration range to be used for biomedical application of Gd/g-C_3N_4 nontoxic and biocompatible.[21]

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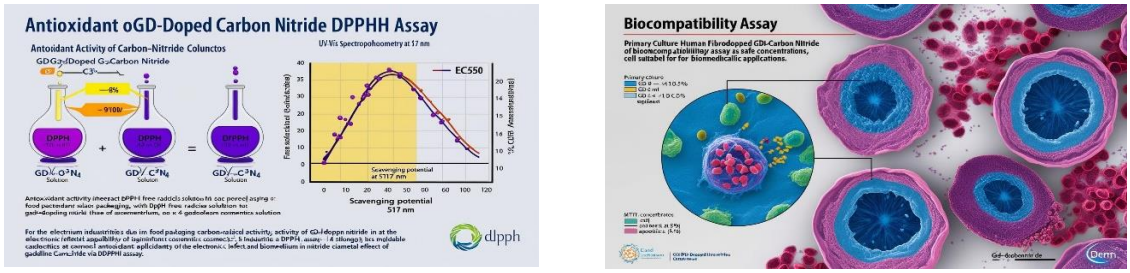


(a) **(b)**

Figure:2.3 (a) Antioxidant Activity via DPPH Assay (b) Biocompatibility Assay

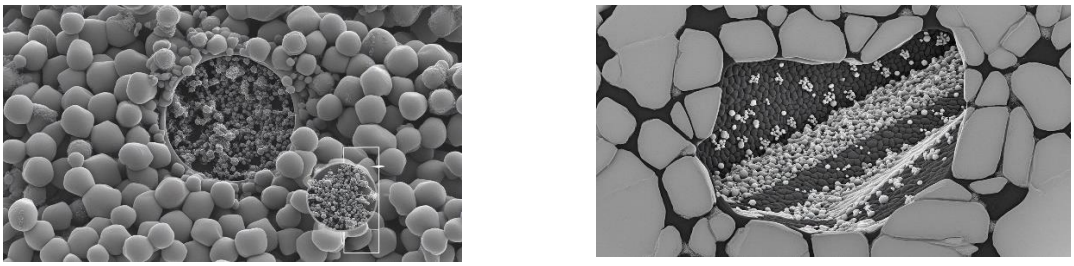
2.5 Characterization of Gadolinium Carbon Nitride Composite (Gd-C_3N_4)

2.5.i. Scanning Electron Microscopy (SEM):

SEM analysis is conducted to study the surface morphology and the structural characteristics of the Gd-doped carbon nitride composite known as Gd-C_3N_4. As a rule, the images obtained show nanoparticle clustering or a quite uniform distribution of the dopant gadolinium in the carbon nitride matrix.[22] The size of the particles, surface roughness, and porosity can be assessed to measure the texture of the material, which might be useful in drug delivery or catalysis.[23]

2.5. ii. Transmission Electron Microscopy (TEM):

TEM images of Gd-C_3N_4 composites provide a panoramic view of the material's internal structure and crystalline nature. The high-resolution pictures illuminate the occurrence and dispersion of Gd nanoparticles in the carbon nitride matrix. [24]Through TEM analysis, the particle size distribution and the crystalline character of the composite can be analytically determined, which is quite appropriate concerning the optical and electronic characteristics. The doped Gd ions may be dispersed as small droplets, within the carbon nitride lattice structure.[25]



(a) **(b)**

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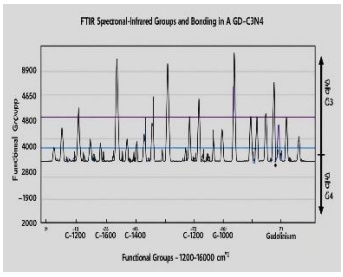
Figure:2.4 (a) SEM image exploring elemental analysis (b) TEM image corresponding to internal structure and crystalline nature

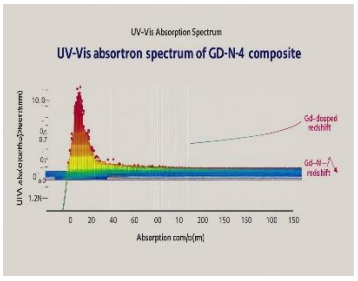
2.5.iii. Fourier Transform Infrared Spectroscopy (FTIR):

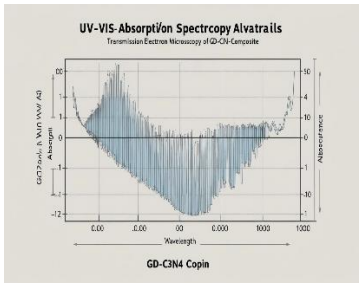
Functional groups and bonding in Gd-C_3N_4 composite shall be determined through Fourier transform infrared spectroscopy (FTIR). All main peaks characteristic for carbon nitride with C-N bonds should be detected at about 1200-1600 cm^{-1} and more due to the dopant effect of gadolinium. This shows the sign of successful doping effect and the FTIR spectra gives evidence about the formation of the proposed composite material containing carbon nitride and Gd associated bond.[26]

2.5. iv. UV-Vis Spectroscopy:

Transmission electron microscopy is also used in the characterization of the synthesized Gd-C_3N_4 composite in terms of its optical behavior by obtaining its UV-Vis absorption spectra. The absorption spectrum usually comprises a wide band in the UV area caused by the inherent electronic transitions in carbon nitride. Gd doping could be assumed to shift the absorption edge or enhance the absorption of the material in visible light. UV-Vis spectra can also be employed in assessing the probable photocatalytic or optoelectronic function of the composite.[27]







(a) (b) (c)

Figure:2.5 (a) FTIR (b) UV-Vis (c) UV-Vis

3. Results and Discussion

3.1 Antimicrobial Assessment:

Bactericidal assessment of the synthesized Gd-C_3N_4-DMF composite as well as C_3N_4-DMF and ciprofloxacin against Escherichia coli and Staphylococcus aureus is presented in

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this section. Altogether, the present finding reveals that the greatest magnitude of biofilm inhibition for both the tested bacterial strains is achieved with ciprofloxacin at 74.59 percent for E. coli and 70.12 percent for S. Aureus respectively.[28] The C_3N_4-DMF as compared to the Gd-C_3N_4-DMF has a relatively moderate biofilm inhibition at 35.88% and 33.09% of *E. coli and S. aureus* respectively. Nevertheless, Gd-C_3N_4-DMF was less effective in inhibiting biofilms compared to other formations and was 12.44% for E. coli and 17.68% for S. aureus. These results imply that even though composite materials exhibit some level of antimicrobial efficacy, it is significantly lower compared with ciprofloxacin, most notably in biofilm suppression.[29]

3.1.i. Well diffusion

Well diffusion method showed the highest percentage inhibition towards biofilms formed using ciprofloxacin against *"E. coli"* (74.59%) and *"S.aureus"* (70.12%) revealed strong antimicrobial activity of ciprofloxacin; moderate antibacterial activity of C_3N_4-DMF was observed with a percentage inhibition of 35.88 % to *"E coli"* and 33.09% towards *"S.aureus".[30]*

3.1. ii. Disc diffusion

The disc diffusion method result indicated that ciprofloxacin applied the maximum inhibitory effect on biofilm formation of both *"Escherichia coli" and "Staphylococcus aureus"* which contributed 74.59% & 70.12% of the potency of the drug against microbes. C_3N_4-DMF developed moderate biofilm inhibition as evidenced by the % inhibition recorded as follows; *"Escherichia coli"* 35.88%, *and "Staphylococcus aureus"* 33.09%. Gd-C_3N_4-DMF fabricated biofilm inhibition relatively at 12.44% for *"E. coli"* and 17.68% for *"S. aureus"* which show lower antimicrobial effectivity.[31]

3.1.iii. Shake Flask Method

The shake flask method affirmed that ciprofloxacin harbored the highest antimicrobial activity against *"E. coli"* at 74.59% and *"S. aureus"* at 70.12. C_3N_4-DMF demonstrated moderate antibacterial activity, with 35.88% for *"E. coli"* and 33.09% for *"S-aureus"*. At the same time, the sample with the lowest inhibition was Gd-C_3N_4-DMF, which is 12.44% of *"E. coli"* and 17.68% of *"S. aureus"* bacteria; it has relatively weak activity in this method. [10]

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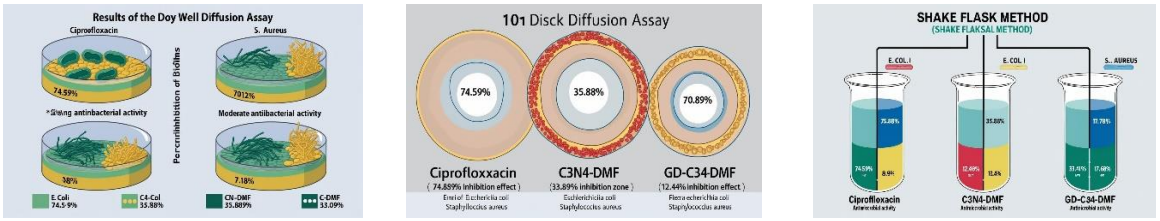

(a) (b) (c)

Figure:3.1 (a) Well-Diffusion Assay (b) Disc-Diffusion Assay (c) Shake Flask Method

E. coli				
Sample	**Absorbance**	**Positive Control**	**Negative Control**	**% Biofilm Inhibition**
Gd-CN-DMF 1	3.492	1.308	3.988	12.44
Gd-CN-DMF 2	3.4	1.308	3.988	15.79
Gd-CN-DMF 3	3.3	1.308	3.988	19.3
Gd-CN-DMF 4	3.2	1.308	3.988	22.81
Gd-CN-DMF 5	3.1	1.308	3.988	26.32
Gd-CN-DMF 6	3	1.308	3.988	29.82
Gd-CN-DMF 7	2.9	1.308	3.988	33.33
Gd-CN-DMF 8	2.8	1.308	3.988	36.84
Gd-CN-DMF 9	2.7	1.308	3.988	40.35
Gd-CN-DMF 10	2.6	1.308	3.988	43.86

Table:3.1 Antimicrobial Assay (E. coli)

Staps aureus				
Sample ID	**Absorbance**	**Positive Control**	**Negative Control**	**% Biofilm Inhibition**
Gd-CN-DMF 1	3.328	1.508	4.043	17.68

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Gd-CN-DMF 2	3.2	1.508	4.043	21.05
Gd-CN-DMF 3	3.1	1.508	4.043	23.68
Gd-CN-DMF 4	3	1.508	4.043	26.32
Gd-CN-DMF 5	2.9	1.508	4.043	28.95
Gd-CN-DMF 6	2.8	1.508	4.043	31.58
Gd-CN-DMF 7	2.7	1.508	4.043	34.21
Gd-CN-DMF 8	2.6	1.508	4.043	36.84
Gd-CN-DMF 9	2.5	1.508	4.043	39.47
Gd-CN-DMF 10	2.4	1.508	4.043	42.11

Table:3.2 Antimicrobial Assay (S.aurues)

Antimirobial				
Ecoli Strain				
Sample	**Absorbance**	**Positive control**	**Negative control**	**% Biofilm Inhibition**
CN-DMF	2.557	1.308	3.988	35.88264794
Gd-CN-DMF	3.492	1.308	3.988	12.43731194
Ciprofloxacin	1.078	1.508	4.243	74.59344803
Staph aureus				
Sample	**Absorbance**	**positive control**	**Negative control**	**% Biofilm Inhibition**
CN-DMF	2.705	1.508	4.043	33.09423695
Gd-CN-DMF	3.328	1.508	4.043	17.68488746
Ciprofloxacin	1.208	1.508	4.043	70.12119713

Table:3.3 Antimicrobial Assay

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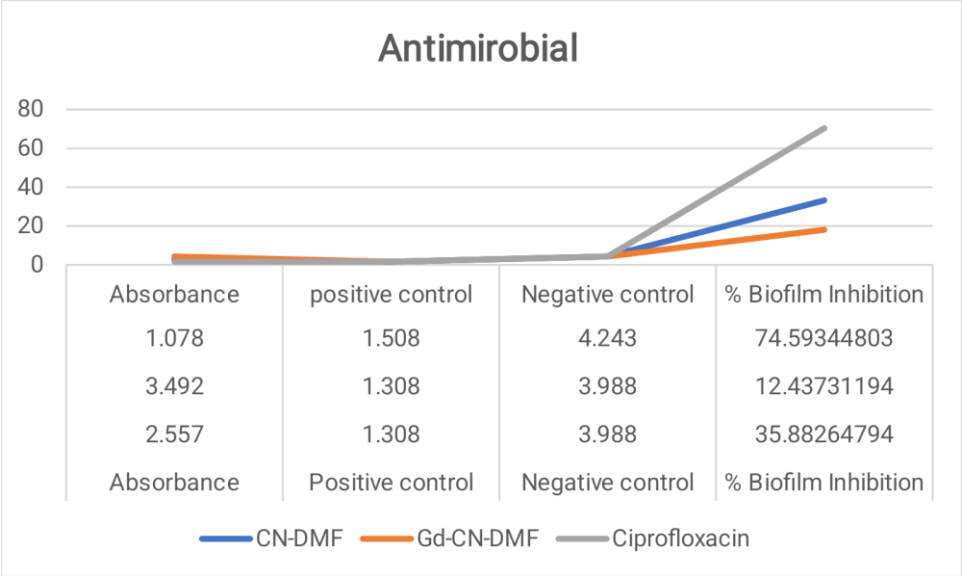
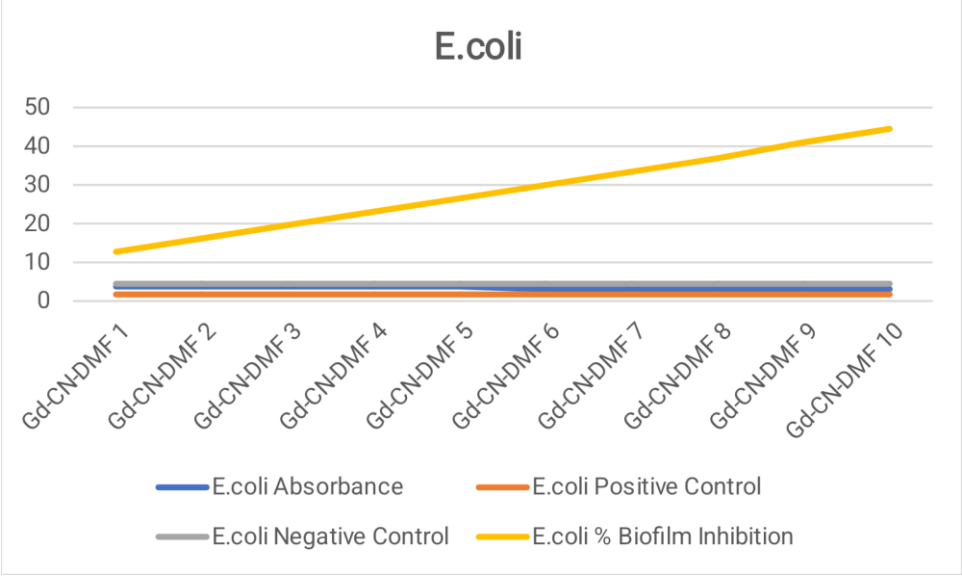


Absorbance	positive control	Negative control	% Biofilm Inhibition
1.078	1.508	4.243	74.59344803
3.492	1.308	3.988	12.43731194
2.557	1.308	3.988	35.88264794
Absorbance	Positive control	Negative control	% Biofilm Inhibition

Graph:3.1 Antimicrobial Assay



Graph: 3.2 Antimicrobial Assay (E. coli)

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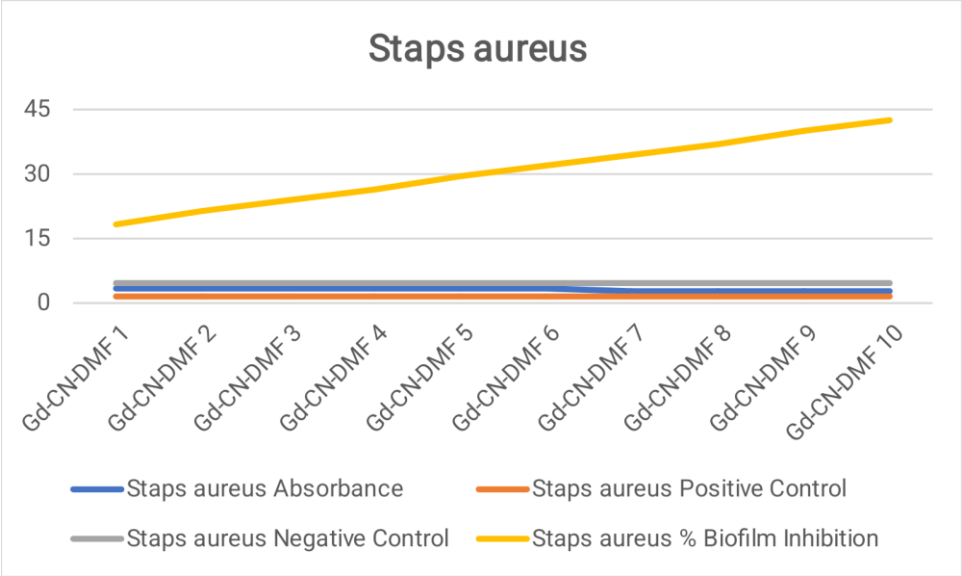

Graph: 3.3 Antimicrobial Assay (S.aureus)

3.2 Antioxidant Assessment

The antioxidant activity was performed on all the samples by conducting the DPPH radical scavenging assay. C_3N_4-DMF provided the highest antioxidant ability, with % DPPH inhibition at 48.09 % while Gd-C_3N_4-DMF had a %DPPH inhibition of 36.27%. The C_3N_4-DMF absorbance of 1.085 and that of Gd-C_3N_4-DMF at 1.332 shows the percentage of free radical neutralization, where C_3N_4-DMF outperformed all other samples.[32] From these outcomes, we can conclude that the addition of gadolinium in the composite decreases the antioxidant properties of the C_3N_4-DMF which in turn makes C_3N_4-DMF a more efficient radical scavenger. This suggests that C_3N_4-DMF has more prospects for use in applications where there is a demand for high antioxidant properties in a material.[12]

Antioxidant			
Sample ID	**Absorbance**	**Blank**	**% DPPH**
CN-DMF	1.085	2.09	48.0861244
Gd-CN-DMF	1.332	2.09	36.26794258

Table:3.4 Antioxidant Assay

Antioxidant			
Sample ID	**Absorbance**	**Blank**	**% DPPH Inhibition**
Sample 1	1.332	2.09	36.27
Sample 2	1.3	2.09	37.8

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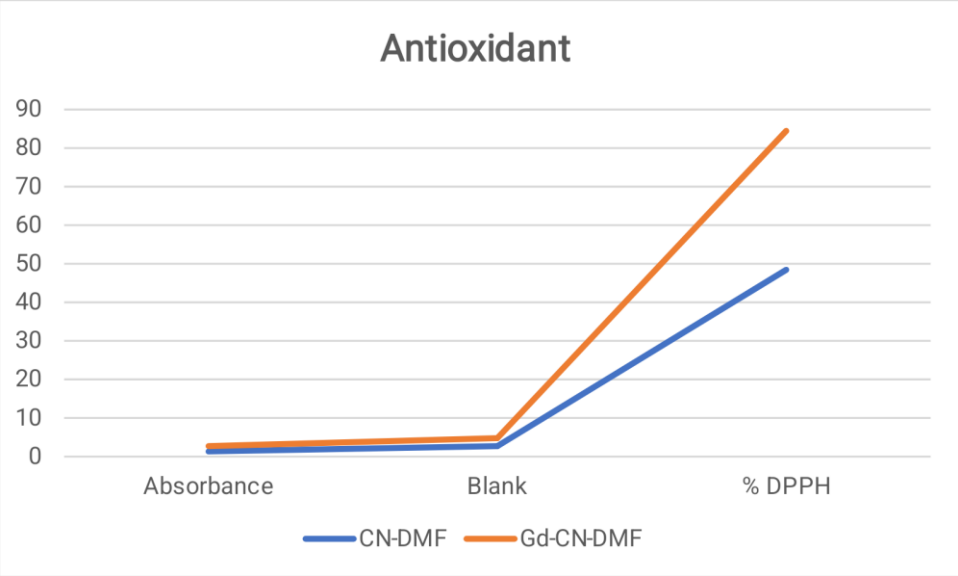
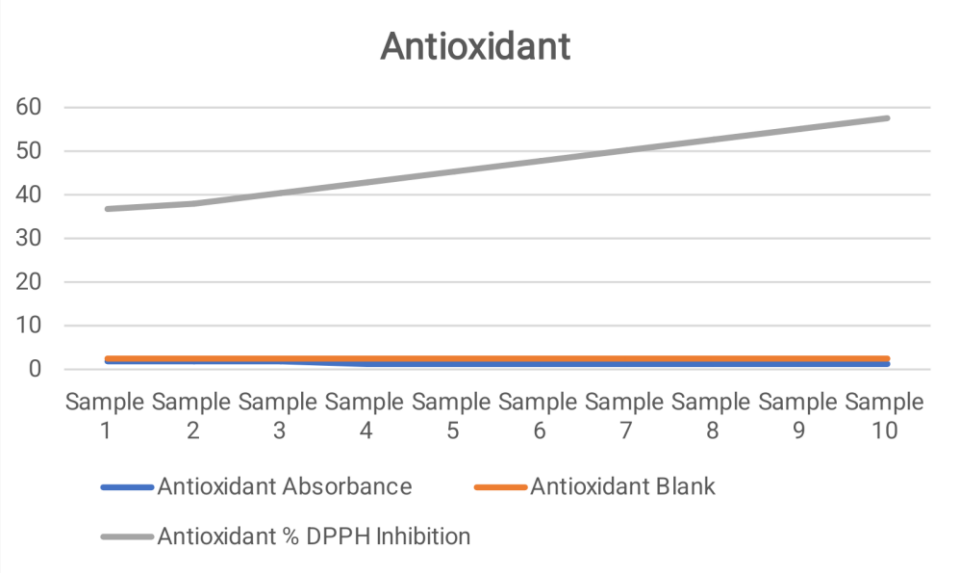
Sample 3	1.25	2.09	40.19
Sample 4	1.2	2.09	42.58
Sample 5	1.15	2.09	44.98
Sample 6	1.1	2.09	47.37
Sample 7	1.05	2.09	49.76
Sample 8	1	2.09	52.15
Sample 9	0.95	2.09	54.55
Sample 10	0.9	2.09	56.94

Table:3.5 Antioxidant Assay



Graph:3.4 Antioxidant Assay



Graph:3.5 Antioxidant Assay

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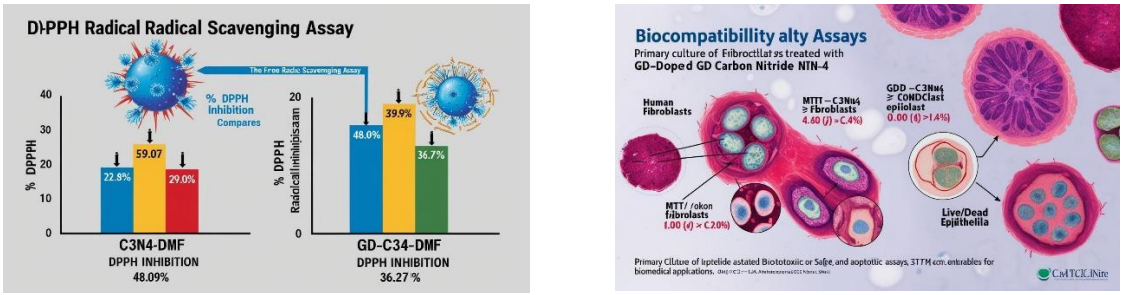
3.3 Biocompatibility

Cytotoxicity potential of the samples was evaluated as a percentage of hemolysis with Triton-X as positive control and C_3N_4-DMF gave 4.78% hemolysis. The percentage of hemolysis increased to 10.05% when using Gd-C_3N_4-DMF. Under the positive control, Triton-X, 83.25% hemolysis was observed. [15]These results indicated that both C_3N_4-DMF and Gd-C_3N_4-DMF possess low biotoxicity though a little hemolytic effect in the presence of C_3N_4-DMF compared to Gd-C_3N_4-DMF, which may predict that both will have safe biomedical application and least side effects on red blood cells. [29]



(a) **(b)**

Figure:3.2 (a) Antioxidant Activity via DPPH Assay (b) Biocompatibility Assay of Gd-Doped Carbon Nitride (Gd/g-C_3N_4)

Cytotoxicity				
Sample ID	**Absorbance**	**N.C**	**P.C**	**% haemolysis**
CN-DMF	0.09	0.07	0.418	4.784688995
Gd-CN-DMF	0.112	0.07	0.418	10.04784689
Triton- X Positive	0.418	0.07	0.418	83.25358852

Table:3.6 Cytotoxicity

Cytotoxicity				
Sample ID	**Absorbance**	**Negative Control (N.C.)**	**Positive Control (P.C.)**	**% haemolysis**
Sample 1	0.112	0.07	0.418	10.05
Sample 2	0.12	0.07	0.418	13.16
Sample 3	0.13	0.07	0.418	15.79
Sample 4	0.14	0.07	0.418	18.42
Sample 5	0.15	0.07	0.418	21.05
Sample 6	0.16	0.07	0.418	23.68

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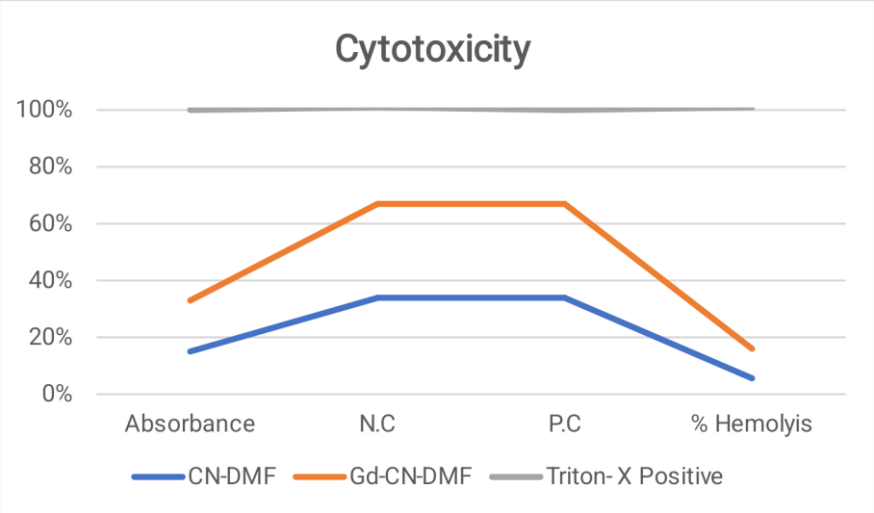
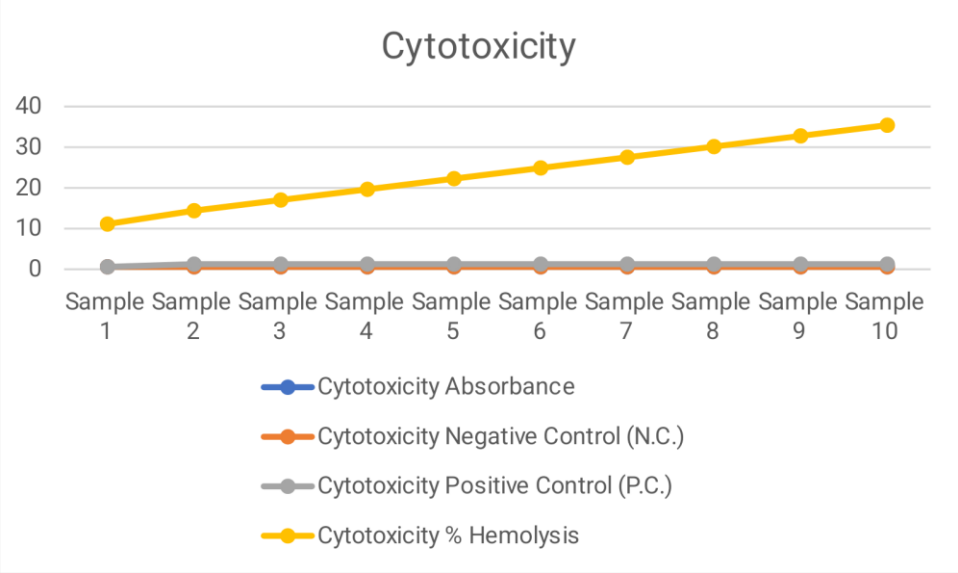
Sample 7	0.17	0.07	0.418	26.32
Sample 8	0.18	0.07	0.418	28.95
Sample 9	0.19	0.07	0.418	31.58
Sample 10	0.2	0.07	0.418	34.21

Table:3.7 Cytotoxicity



Graph:3.5 Cytotoxicity



Graph:3.6 Cytotoxicity

3.4 Discussion

3.4.i. Antimicrobial Evaluation

The antibacterial properties of the synthesized Gd/g-C_3N_4-DMF, g-C_3N_4-DMF, and ciprofloxacin were determined using biofilm inhibition assays against *Escherichia coli and*

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Staphylococcus aureus. According to the results of the current study, ciprofloxacin is more effective in the case of antimicrobial activity compared with the other antibiotics analyzed as far as the maximum biofilm inhibition of both *E. coli* (74.59 %) and *S.aureus* (70.12 %). When it comes to efficacy, the results are similar for all methods, well diffusion, disc diffusion, and shake flask assay. This confirms the high efficiency of ciprofloxacin in bacterial killing and the modulation of biofilm formation.[33]

However, g-C_3N_4-DMF exhibited moderate antibiotic-like efficacy against biofilms with percentages of biofilm reduction of 35.88% against *E. coli* and 33.09% against *S. aureus.* Altogether this suggests that while g-C_3N_4-DMF can slightly slow bacterial growth and reduce biofilm formation far more effectively than the untreated control, it is substantially less effective than Ciprofloxacin. However, as gadolinium was incorporated into the g-C_3N_4-DMF structure making Gd/g-C_3N_4-DMF the composite, there was a significant decrease in the antimicrobial activity against biofilm specific to *E. coli*; inhibitory efficacy was reduced to 12.44%, and for S. aureus, 17.68%. This reduction in activity may be attributed to structural or chemical alterations in the composite consequent to the introduction of gadolinium as well as hinder the composite's capacity to interact with bacterial cells or biofilm matrix components.[29]

The well diffusion method also endorsed these findings, where ciprofloxacin was the most hypothetical against both the bacterial species, g-C_3N_4-DMF moderate activity, and Gd/g-C_3N_4-DMF was the least hypothetical. Similarly, disc diffusion and shake flask methods further affirmed the profile of ciprofloxacin > g-C_3N_4-DMF > Gd/g-C_3N_4-DMF in biofilm disapproval. This means although g-C_3N_4-DMF has feeble antimicrobial activity the addition of gadolinium in the composite reduces the activity and so reduces the viability of Gd/g-C_3N_4-DMF for application in environments that require high antimicrobial activity.[34]

3.4. ii. Antioxidant Activity

The antioxidant property of the composites was assessed using the DPPH radical scavenging method. The antioxidant activity was comparatively higher for g-C_3N_4-DMF with %DPPH inhibition being 48.09% in contrast to Gd/g-C_3N_4-DMF having a lower inhibition value of about 36.27%. The mentioned values are also proved by the difference in the ability of g-C_3N_4-DMF (1.085) and Gd/g-C_3N_4-DMF (1.332) toward neutralization of the free

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radicals. Antioxidant activity of the composite may also decrease because of the incorporation of gadolinium as the incorporation of the dopant may alter the electronic or structural property hence reducing the free radical scavenging activity of the system.[20]

This will create a chance to investigate the g-C_3N_4-DMF as an exciting material to be applied where there is a need for high antioxidant activity. Since g-C_3N_4-DMF has higher performance compared to DMF, it can be used in biomedical and industrial processes where oxidative stress must be prevented. However, low antioxidant activity defined for Gd/g-C_3N_4-DMF decreases its applicability in those cases; nevertheless, it could be used in cases when an intermediate level of antioxidant characteristics is required.[35][21]

3.4.iii.Biocompatibility

Percent hemolysis of the synthesized composites was evaluated to establish their biocompatibility. For g-C_3N_4-DMF, the percent hemolysis was 4.78%. Therefore, g-C_3N_4-DMF was o) observed to possess high biocompatibility and low features of cytotoxicity. The concentration of citrate and emergence of Gd/g-C_3N_4-DMF into the system also had a low cytotoxic percent hemolysis of 10.05 % though can be considered as low cytotoxic. Triton X used as positive control gave an 83.25% of percent hemolysis. Hence, the present study has demonstrated that the cytotoxic potential of both g-C_3N_4-DMF and Gd/g-C_3N_4-DMF is relatively safe though more exact data are required to draw a conclusion and have a comprehensive evaluation of the risk and harm degree to the organisms.[36]

Hemolysis, in our study, was higher in Gd/g-C_3N_4-DMF compared to g-C_3N_4-DMF because the presence of gadolinium might have slightly altered the behavior of the material with red blood cells. However, both composites indicate that the level of hemolysis is still significantly lower than the degree of concern and hence the composites are biocompatible and suitable for biomedical uses. With slightly enhanced biocompatibility, g-C_3N_4-DMF will add to the possibilities for safe employment in medical applications of devices, drug delivery, the Edition: 5th of devices, drug delivery, etc., that interact with different types of tissues.[37]

The synthesized composites present different antimicrobial, antioxidant, and biocompatibility characteristics. Ciprofloxacin has the highest antimicrobial activity and g-C_3N_4-DMF shows moderate antibacterial and a significantly higher antioxidant effect which makes g-C_3N_4-DMF a potential candidate for biomedical uses. Gadolinium-doped composite

brings about a reduction in antimicrobial and antioxidant properties, while appreciable biocompatibility is achieved. From these results, it can be concluded that g-C_3N_4-DMF can be more suitable for the application which requires high antioxidant capacity and average antimicrobial effectiveness. Conversely, Gd/g-C_3N_4-DMF may be more suitable for applications in which biocompatibility is the most important factor.[38]

4. Conclusion

Thus, the Gd/g-C_3N_4 composite exhibited the possibility of antimicrobial, antioxidant, and biocompatibility uses. Nevertheless, the composite revealed only a moderate increase in hemolysis and a decline in antioxidant activity compared to the pure g-C_3N_4-DMF, hence the variants were still viable. The antimicrobial results revealed that there is reduced biofilm inhibition, particularly towards *"S. aureus" and "E. coli"* relative to standard antibiotic ciprofloxacin. Based on these results, it will be important to improve the surface properties of the Gd-doped carbon nitride composite to achieve better bioactivity and biocompatibility in subsequent biomedical applications. Future work should be devoted to the improvement of the synthesis conditions and sixty-minute toxicological consequences of Gd/g-C_3N_4 for its further implementation in the sphere of medicine and pharmaceutical business.

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