Eaton M
Cardiff University
School of Engineering

Ward B
Cardiff University
School of Computer Science and Informatics

Maillard JY
Cardiff University
School of Engineering

Zare S
Cardiff University
School of Engineering

ADVANCED MANUFACTURING

Developing Antibacterial Nanocomposites

After the pandemic, we became more aware of microorganisms around us and realized how these creatures could affect our health and environment. In this regard, this project was planned to prepare polymer-based nanocomposites with antibacterial properties. This study selected a cationic polymer, poly (diallyl dimethylammonium) chloride (PDDA), along with graphene oxide reinforcement to achieve this goal. The mechanical and thermal properties of PDDA, GO, and the prepared PDDA-GO nanocomposite will be characterized by dynamic mechanical analysis (DMA) and differential scanning calorimetry (DSC). A high glass transition (Tg) (around 250℃) has been identified with DSC and DMA for the PDDA. The minimum inhibitory concentration (MIC) technique was used to study their antimicrobial activities against Staphylococcus aureus and Escherichia coli. The early results for the PDDA were shown to be moderately effective against E. coli and S. aureus, with values of 64 and 16 µg/mL, respectively.

Keywords:
Nanocomposites, PDDA, antibacterial, graphene oxide, cationic polymer.

Corresponding author:
EatonM@cardiff.ac.uk

M. Eaton, J.Y. Maillard, B. Ward, and S. Zare, 'Developing Antibacterial Nanocomposites', *Proceedings of the Cardiff University Engineering Research Conference 2023*, Cardiff, UK, pp. 105-108.

doi.org/10.18573/conf1.y

INTRODUCTION

Over the past decades, nanomaterials and polymer nanocomposites have gained massive engagement in various fields; For instance, biomedical applications [1], sensors and biosensors [2], and tissue and scaffold engineering [3]. Materials at the nanometre scale permit the designing and generating of new products with outstanding performance and enhanced characteristics [4].

Nanocomposites have a solid structure in which the distance between the phases includes at least a dimension with nanoscale size [5]. Composites generally have two "matrix" and "reinforcement" phases. There are three prevalent matrices, polymer, metal, and ceramic, with reinforcement in various forms such as lamina, fillers, fibre (short and continuous), flake, and particles.

Herein, we aim to prepare polymer-based with reinforcing graphene oxide nanocomposites with potential antimicrobial properties. Polymers as nanocomposite matrices can have almost the same mechanical and thermal properties as metal and ceramics matrices with easier and more affordable processing [6].

With the development of nanotechnology, novel nanomaterials have been proposed with considerable antimicrobial activity, especially in the nanocomposite forms [7]. Nanomaterials like graphene and its derivatives, oxide nanoparticles, carbon nanotubes, etc., have been used as fillers in nanocomposites with incredible outcomes [8].

Among them, graphene oxide (GO), Due to the presence of these functional groups such as epoxy, carbonyl and hydroxyl functional groups, is the most accustomed one and can be dispersed in water, which caused it to be favoured for biomedical applications [9].

Moreover, graphene oxide, has been successfully used as a reinforcement for polymers owing to its outstanding thermal, mechanical, and electrical properties. The exfoliation and good dispersion of GO, along with a strong interface interaction between GO and polymer, are crucial to observe such property enhancements [10].

Furthermore, graphene oxide, due to its outstanding thermal, mechanical and electrical properties, has been successfully used as a reinforcement for polymers. The deep interface interaction between GO and polymer, along with its exfoliation and good dispersion, are crucial to observe such property enhancements.

Moreover, owing to the exfoliation and good dispersion of GO, along with a strong interface interaction between GO and polymer, it has been successfully used as a reinforcement for polymer nanocomposites, along with outstanding thermal, mechanical, and electrical properties.

Numerous studies have been conducted on the potential antimicrobial activity of GO. The antibacterial property has been associated with the physical damage to cell membranes due to membrane stress which has been caused by sharp edges of the GO, Which results in leakage of RNA from bacteria membrane; Additionally, the oxidative stress has been identified as the most widely accepted mechanism via either a ROS-dependent or a ROS-independent pathway, which can interfere with bacterial metabolism and disrupt crucial cellular functions, eventually leading to inactivation or even cell death [11]. It was suggested that graphene may induce oxidative stress on neural phaeochromocytoma-derived PC12 cells [12]. Several research has been conducted on this antibacterial activity of GO, and inconclusive results have been reported. A recent report has shown that contact of E. coli and Staphylococcus aureus bacterial cells with GO can result in growth reductions of about 51 and 61%, respectively [13].

Given the above, the initial objective of the presented work was to identify a suitable polymer with antimicrobial properties. One of the most promising candidates is polycationic family of polymers, this is due to their mass and charge localization that promotes electrostatic interaction between the cationic structure and the negatively charged bacterial cell membrane, causing disruption of the cell membrane and/or wall, and reaction with the cytoplasmic membrane (lipid or protein) followed by membrane disorganization [14]. Amongst polytcationic polymers, quaternary ammonium compounds (QACs) are the most valuable antiseptics and disinfectants. Furthermore, they are environment friendly which favours their usage [15]. Between all available QACs, poly (diallyl dimethylammonium) chloride (PDDA) was chosen in this study as it displayed an outstanding antimicrobial activity [16].

MATERIALS AND METHODS

Materials
PDDA (20% water solution, average Mw = 100,000– 200,000) was purchased from Sigma Aldrich. Graphene oxide (1% aqueous dispersion, 1L) was acquired from GOgraphene and used as received. Distilled water (DI) was used in all the fabrication process, including solutions preparation and washing.

Preparation of PDDA-graphene oxide nanocomposite.
Graphene oxide sheets were functionalized with PDDA by non- covalent bonding by adding suitable amounts of 1 wt% GO to 20 wt% PDDA aqueous solutions and sonicated for half an hour.

Antibacterial Activity
In order to study the antimicrobial properties of the antimicrobial agents (PDDA and GO) and the prepared nanocomposite, the minimum inhibitory concentration (MIC) method was used. Two strains including Gram-negative *E. coli* ATCC 25922 and Gram-positive *S. aureus* ATCC 6538 were selected for antibacterial tests because they are usually associated with the medical-associated infections. The target *E. coli* and *S. aureus* cultures were grown overnight in a Luria– Bertani (LB) broth at 37 °C. The cultures were subsequently diluted in fresh medium and grown until 2h, which was confirmed by measuring the optical density at 600 nm. Afterward, the bacterial cells were suspended and diluted in sterile saline solution to 10^7 colony-forming units (CFU) mL^{-1}.

For the MIC tests, the antibacterial agents were diluted to serial dilutions (1028, 512, 256, 128, 64, 32, 16, 8, 4, 2 µg mL^{-1}) by sterile saline solution before being inoculated with the bacteria suspension. The lowest concentration that can satisfied, represented the MIC of the antibacterial materials.

Thermal and Mechanical properties
The thermal decomposition, curing temperature, and the glass transition temperature (Tg) of PDDA, GO, and the PDDA-GO nanocomposite were measured by differential scanning calorimetry (DSC) (Netzsch / DSC 200) instrument in the nitrogen atmosphere. For DSC analysis, specimens were heated with continuous increment in temperature from -80 to 360°C with a rate of 20°C/min.

Perkin Elmer DMA 8000 instrument at dual cantilever clamp mode at 1 Hz and 10 HZ of vibration frequency was used to measure the dynamic mechanical property and thermal properties of the PDDA, GO, and the PDDA-GO nanocomposite. The dynamic force was applied in the temperature from 30 to 360°C with heating rate 3°/min.

RESULTS AND DISCUSSION

Antibacterial Activity of PDDA.
The minimum inhibitory concentration (MIC) as a reference method, have been widely used to test the in-vitro antibacterial activity of antimicrobial agents that inhibits the visible and rapid growth of the bacteria for measuring the potential agents against specific pathogens. Target pathogens were chosen *E. coli* as Gram-negative bacteria, *S. aureus* as Gram-Positive bacteria. As displayed in the polymer activity after an overnight incubation toward both kinds of bacteria was examined for three times. Results showed PDDA was effective against both bacteria in a concentration-dependent manner values for *E. Coli* and *S. aureus* of 16 and 64 ug/ml, respectively. These observed MIC values are suggesting that the PDDA has antibacterial activity in concentrations from 16 (against *E. coli*) and 64 µg mL^{-1} (against *S. aureus*).

Bacteria	PDDA concentration (µg mL^{-1})
E. coli	>16
S. aureus	>64

Table 1. Antibacterial activity of PDDA.

Thermal properties of PDDA
To investigate the thermal properties of PDDA, the DSC (Differential Scanning Calorimetry) was first used to determine the glass transition (Tg) and the polymer's melting temperature.

Various weights and heating ranges were used to do the DSC analysis. Still, we have not found the exact data for the Tg and melting point of PDDA and there has not been any related data online to compare our results with them. But after trying multiple times the latest results are as shown in Fig. 1. DSC graph of PDDA.

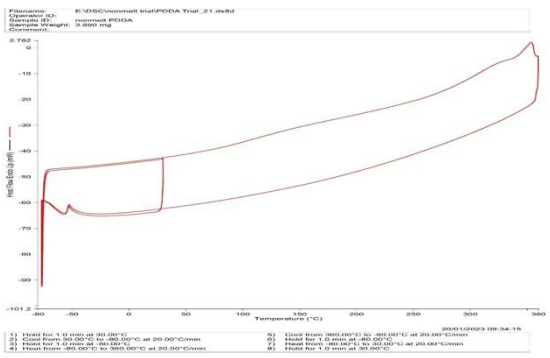

Figure 1. DSC graph of PDDA.

As can be seen, there is a peak around 360°C, which suggests the melting point. However, usually seeing a melting point on the DSC should give us a mutual peak on the other side of the spectrum as the crystallization peak. Since we cannot see that, we are uncertain to say this is the exact melting point. Also, Tg usually shows a slight shift. There is a slight shift around 100°C, but we consider that as water vaporizing from the polymer since PDDA is super water absorbent, and this shift changes each time with different samples using.

For further investigation, DMA (Dynamic Mechanical Analysis) has been used. This technique is usually used to study a polymer's viscoelastic behavior by varying the temperature of the sample or the frequency of the stress applied to it. The analysis can give two graphs, the "modulus", which measures the material's overall resistance to deformation and the "tan delta", measuring the material's damping. We can determine the glass transition and melting point based on these two graphs.

The analysis was done at two frequencies (1 Hz and 10 Hz). The glass transition is frequency dependent; therefore, as seen in the Figure 2, the 1Hz and 10Hz modulus graphs have separated around 250°C. Also, the tan delta signal is the point in the transition region where the material has the most viscous response to deformation. This signal often shows a distinct peak in the transition region, and the Tg measured from the tan(δ) is simply the temperature at the peak. As shown in the Figure 3 this peak is also around 250°C suggesting the Tg of PDDA. Also, on continued heating, the melting point, is reached around 360°C both in modulus and tan delta graph suggesting the melting point which is the same result we seen in the DSC analysis graph.

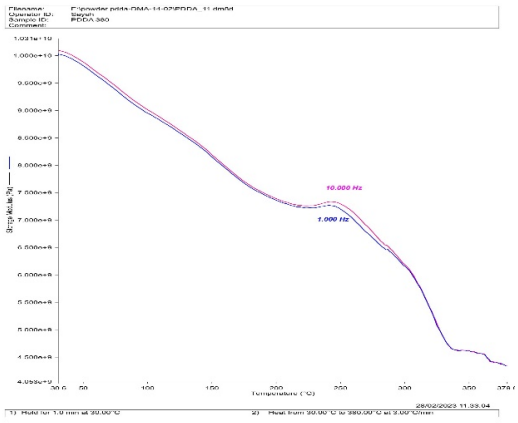

Figure 2. DMA of PDDA (the storage modulus graph).

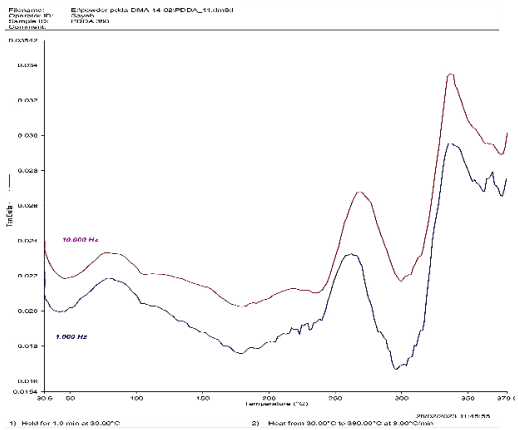

Figure 3. DSC of PDDA (the tan delta graph).

CONCLUSIONS AND FUTURE WORK

From these primary results it can be concluded that the PDDA has a high temperature melting point, which will cause problem for the process of our nanocomposite. Therefore, now we are mainly looking at how to shift these high temperatures to lower by adding additives to the polymer.

Conflicts of interest
The authors declare no conflict of interest.

REFERENCES

[1] C. Sharma and N.K. Bhardwaj, 'Bacterial nanocellulose: Present status, biomedical applications and future perspectives' *Materials Science and Engineering: C*, Vol. 104, pp. 109963, 2019. doi.org/10.1016/j.msec.2019.109963

[2] S. Hameed and P. Bhattarai, 'Novel Nanomaterials for Biosensor Development', *Nanobiosensors: From Design to Applications,* pp.45-72, 2020. doi.org/10.1002/9783527345137.ch3

[3] J. Mahendran and J.-P. St-Pierre, 'Nanomaterials Applications in Cartilage Tissue Engineering', *Nanoengineering Materials for Biomedical Uses,* pp. 81-105, 2019. doi.org/10.1007/978-3-030-31261-9_5

[4] M. B. Ross, P. De Luna, Y. Li, C.-T. Dinh, D. Kim, P. Yang, *et al.*, 'Designing materials for electrochemical carbon dioxide recycling', *Nature Catalysis*, Vol. 2, Issue 8, pp. 648-658, 2019. doi.org/10.1038/s41929-019-0306-7

[5] M.R. Rahimi and S. Mosleh, *Intensification of Sorption Processes: Active and Passive Mechanisms*. Elsevier, 2021.

[6] M. Roy, P. Tran, T. Dickens and A. Schrand, 'Composite Reinforcement Architectures: A Review of Field-Assisted Additive Manufacturing for Polymers', *Journal of Composites Science*, vol. 4, no. 1, p. 1, 2019. doi.org/10.3390/jcs4010001

[7] M. Azizi-Lalabadi, H. Hashemi, J. Feng, and S. M. Jafari, 'Carbon nanomaterials against pathogens; the antimicrobial activity of carbon nanotubes, graphene/graphene oxide, fullerenes, and their nanocomposites', *Advances in Colloid and Interface Science*, vol. 284, p. 102250, Oct. 2020. doi.org/10.1016/j.cis.2020.102250

[8] O. Movil, L. Frank, and J. A. Staser, 'Graphene Oxide–Polymer Nanocomposite Anion-Exchange Membranes', *Journal of The Electrochemical Society*, vol. 162, no. 4, pp. F419–F426, 2015. doi.org/10.1149/2.0681504jes.

[9] L. Hui e*t al.*, 'Availability of the Basal Planes of Graphene Oxide Determines Whether It Is Antibacterial', ACS *App Mater Interfaces*, vol. 6, no. 15, pp. 13183–13190, Aug. 2014. doi.org/10.1021/am503070z

[10] M. Cobos, I. De-La-Pinta, G. Quindós, M. J. Fernández, and M. D. Fernández, 'One-step eco-friendly synthesized silver-graphene oxide/poly(vinyl alcohol) antibacterial nanocomposites', *Carbon*, vol. 150, pp. 101–116, Sep. 2019. doi.org/10.1016/j.carbon.2019.05.011.

[11] X. Zou, L. Zhang, Z. Wang, and Y. Luo, 'Mechanisms of the Antimicrobial Activities of Graphene Materials', *J Am Chem Soc*, vol. 138, no. 7, pp. 2064–2077, Feb. 2016. doi.org/10.1021/jacs.5b11411

[12] S. Liu et al., 'Antibacterial Activity of Graphite, Graphite Oxide, Graphene Oxide, and Reduced Graphene Oxide: Membrane and Oxidative Stress', *ACS Nano*, vol. 5, no. 9, pp. 6971–6980, Sep. 2011. doi.org/10.1021/nn202451x

[13] Q. Bao, D. Zhang, and P. Qi, 'Synthesis and characterization of silver nanoparticle and graphene oxide nanosheet composites as a bactericidal agent for water disinfection', *Journal of Colloid and Interface Science*, vol. 360, no. 2, pp. 463–470, Aug. 2011. doi.org/10.1016/j.jcis.2011.05.009

[14] L. D. Melo, E. M. Mamizuka, and A. M. Carmona-Ribeiro, 'Antimicrobial Particles from Cationic Lipid and Polyelectrolytes', *Langmuir*, vol. 26, no. 14, pp. 12300–12306, Jul. 2010. doi.org/10.1021/la101500s

[15] B. Issa, I. M. Obaidat, B. A. Albiss, and Y. Haik, 'Magnetic Nanoparticles: Surface Effects and Properties Related to Biomedicine Applications', *International Journal of Molecular Sciences*, vol. 14, no. 11, pp. 21266–21305, Nov. 2013. doi.org/10.3390/ijms141121266

[16] L. M. Sanches, D. F. S. Petri, L. D. De Melo Carrasco, and A. M. Carmona-Ribeiro, 'The antimicrobial activity of free and immobilized poly (diallyldimethylammonium) chloride in nanoparticles of poly (methylmethacrylate)', *Journal of Nanobiotechnology*, vol. 13, no. 1, p. 58, Dec. 2015. doi.org/10.1186/s12951-015-0123-3

Cynhadledd Ymchwil Peirianneg Caerdydd
Cardiff Engineering Research Conference

E. Spezi and M. Bray (eds.) 2024. *Proceedings of the Cardiff University Engineering Research Conference 2023.* Cardiff: Cardiff University Press.
doi.org/10.18573/conf1

Cardiff University Engineering Research Conference 2023 was organised by the School of Engineering and held from 12 to 14 July 2023 at Cardiff University.

The work presented in these proceedings has been peer reviewed and approved by the conference organisers and associated scientific committee to ensure high academic standards have been met.

Cardiff University Press | Gwasg Prifysgol Caerdydd

First published 2024

Cardiff University Press
Cardiff University, PO Box 430
1st Floor, 30-36 Newport Road
Cardiff CF24 0DE

cardiffuniversitypress.org

Editorial design and layout by
Academic Visual Communication

ISBN: 978-1-9116-5349-3 (PDF)