Surface interaction studies of novel 2D materials with gram negative and gram-positive pathogens and an enveloped virus

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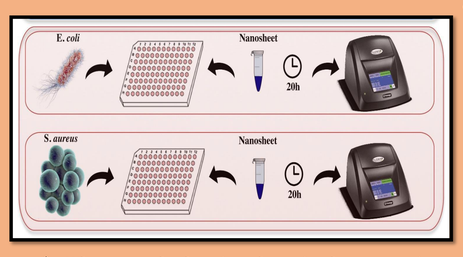
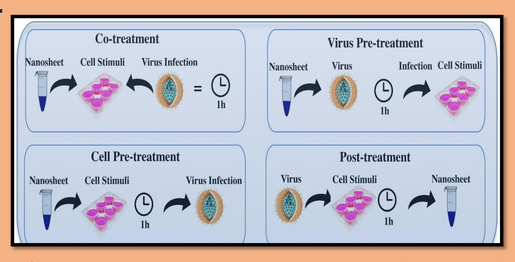
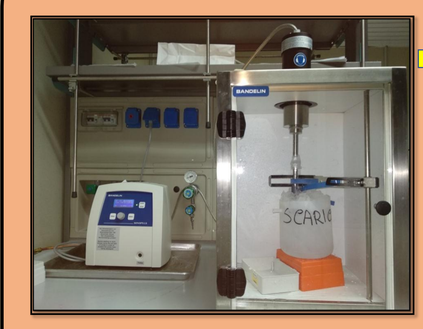
Abstract



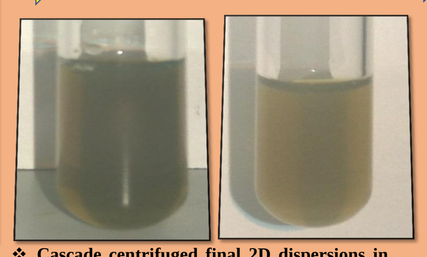


- The novel physio-chemical properties of 2D materials are the driving force to exhibit their anti-bacterial and anti-viral actions.
- **The current study represents the interaction between a gram-negative bacterium, *Escherichia coli*, and a gram-positive bacterium, *Staphylococcus aureus*, with two different types of 2D nanoflakes such as MoS_2, belonging to the Transition Metal Dichalcogenides family, and Graphene Oxide exfoliated in water only.**
- The same two types of nanomaterials were employed to study their antiviral action toward the Herpes simplex virus type-1, (HSV-1). The experimental results showed different bactericide impacts as well as different antiviral power between the two nanomaterials. .

Material fabrication



- **Liquid phase exfoliation of 2D MoS_2 and graphite oxide nanosheets, novel fabrication route to form biofilms as well.**
- **Optimized parameters results in stable dispersions up to three weeks in water.**



- **Cascade centrifuged final 2D dispersions in pure water having stability up to three weeks; centrifuged at 1000g and 2000g for 60 minutes each.**
- **Obtained concentration of the final dispersion was in the range of 200-250 µg/mL.**

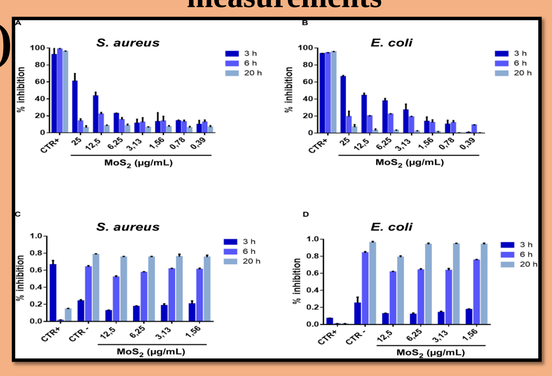
Centrifugal force	MoS2 nanosheets	Graphene oxide nanosheets
1000g	-25.6 ± 0.7	-46.9± 1.5
2000g	-29.2 ± 1.3	-48± 1.2
3000g	-23.4± 0.4	-47.5± 0.7

ζ-Potential values of MoS_2 and graphene oxide nanosheets dispersion at different centrifugal forces.

2D Material- Bacteria surface interaction

Bacterial growth inhibition measurements

1)



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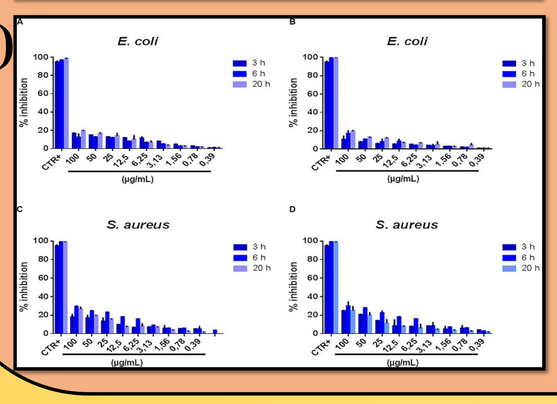


(Antibacterial effect of MoS_2 nanosheets)

- **Bacterial growth inhibition is represented at 3, 6 and 20 h of treatment duration; significant antibacterial effect is observed at 25 µg/mL.**
- **In 1) A-B, the anti bacterial effect decreases upon increasing the incubation time; at 20 h of incubation the bactericide effect is saturated.**
- **In 1) C-D, the bacterial inhibition is studied in different broth mediums; upon increasing the incubation period the antibacterial effect is significantly reduced because of the presence of different ions in the medium.**
- **Exposed sulfur layers and membrane stress accounts for the cytotoxic behaviour towards the bacteria.**

	E. coli			*S. aureus*		
	Damaged	Not Damaged	% Damaged	Damaged	Not Damaged	% Damaged
Figures 5C,D				**Figures 3A,B**		
3 h incubation	64*	11*	85%	2*	/	100%
3 h incubation	38*	7*	84%	2	/	100%
Figure 5E				**Figures 3G,H**		
6 h incubation	55*	3*	91%	86*	41	68%
6 h incubation	46*	2*	96%	90*	50*	64%

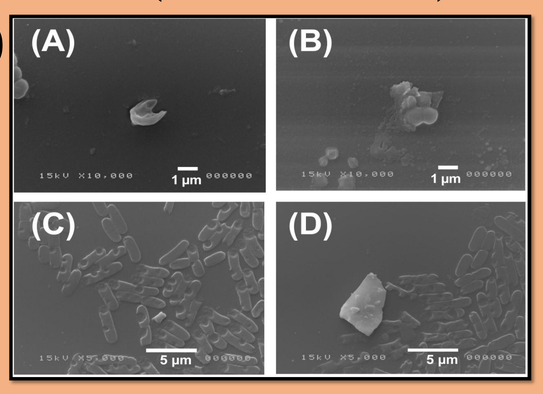
*It represents the statistical data which quantifies the interaction of MoS_2 NSs with E. coli and S. aureus for 3 and 6 h incubation. *Image with MoS_2 flakes.*

(Antibacterial effect of graphene oxide nanosheets)

- **Lower bacterial inhibition is observed at shorter incubation period; 3 and 6h even at the highest concentration of 100 µg/mL as seen in 3) A-D.**
- **Linear increase of the antimicrobial action with the graphene oxide nanosheets concentration 20% and 30% for *E. coli* and *S. aureus* respectively.**
- **Graphene oxide (1) exhibits 200 nm lateral size and 1 nm thickness whereas, Graphene oxide (2) exhibits 400 nm lateral size and 1.5 nm thickness.**

Morphological damage to the bacterium (*E. coli and S. aureus*)

2)



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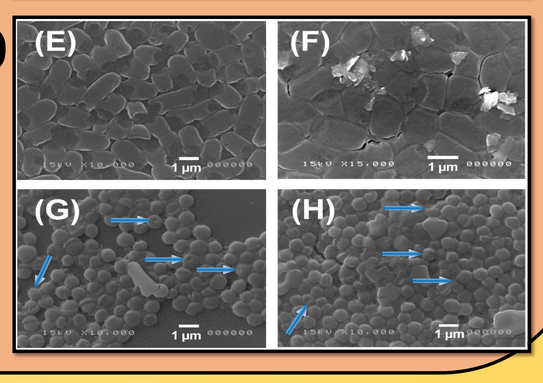
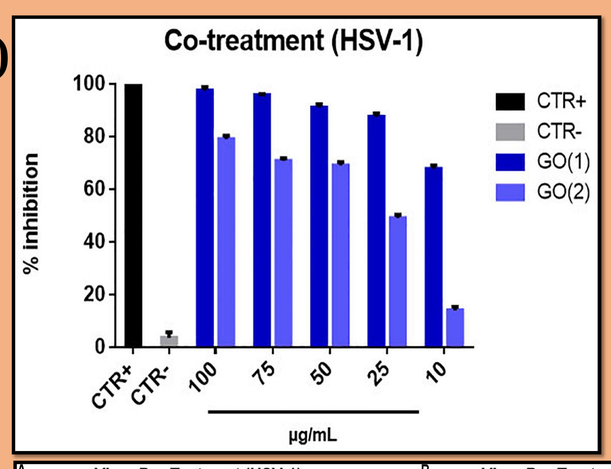


2D Material- Virus surface interaction

Virus and cells treatment assays

1)



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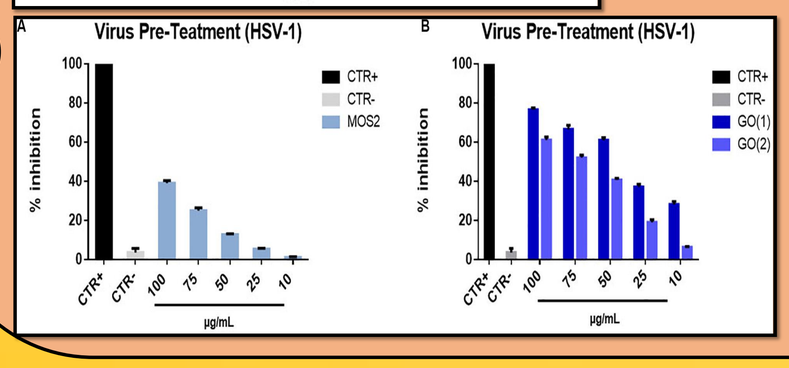
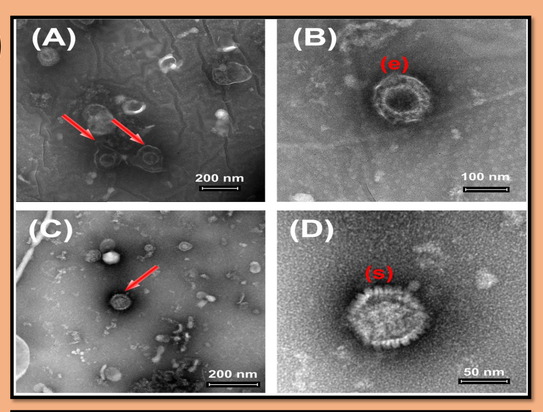


(Antiviral effect of MoS_2 and GO nanosheets)
GO NSs were potent antiviral agent than MoS_2 NSs.

- **Virus Pre-treatment case- Moderate antiviral action by MoS_2 NSs and robust effect by GO NSs.**
  - **Antiviral action-** For MoS_2 NSs, that have an average later size of 150 nm with an average thickness of 1.2 nm, we reached a maximum inhibition of about 40% for the highest NSs concentration of 100 mg/mL.
  - Whereas the antiviral action reaches its maximum at about 75 and 65% inhibition for 100 mg/mL concentration for the two different types of GO NSs, GO(1) GO(2), respectively.
- **Co-treatment case- Very intriguing and surprising finding was; No effect was observed by MoS_2 NSs in comparison with a strikingly strong effect by GO NSs, an antiviral action even stronger than for the virus pre-treatment case.**
  - **Antiviral action-** The MoS_2 NSs can likely be functionalized in the medium by acquiring protons, i.e., H+ ions, on their edges rich of sulfur atoms content, thus forming thiol groups. These groups then are highly repelled by the Vero cell membranes, which have -HS groups on their surface).
  - Essentially, the mechanism is like what described for the virus pre-treatment case, but much more efficient now, MoS_2 nanoflakes are strongly repelled and going to the opposite direction.
- **Cell Pre-treatment and Post-treatment case- No antiviral action was observed at for both MoS_2 and GO NSs.**

Morphological damage to the HSV-1 Virus

2)



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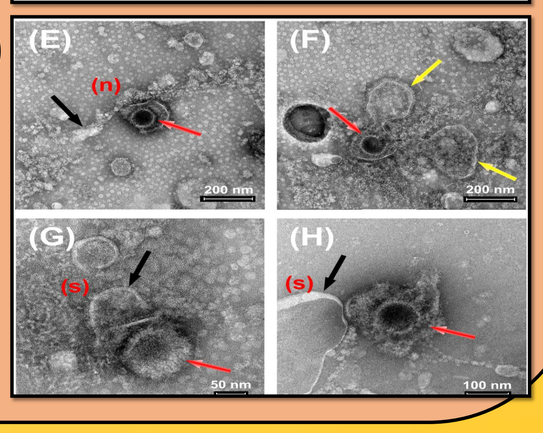
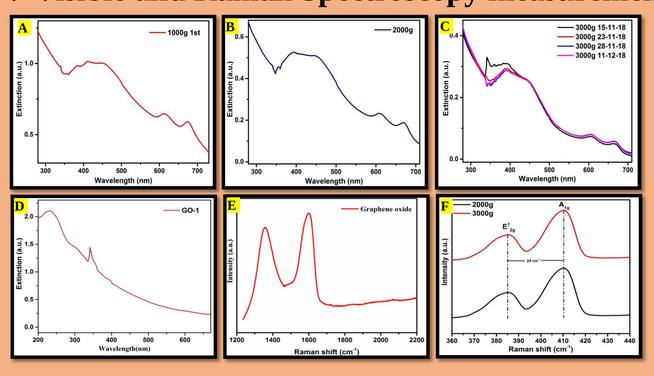


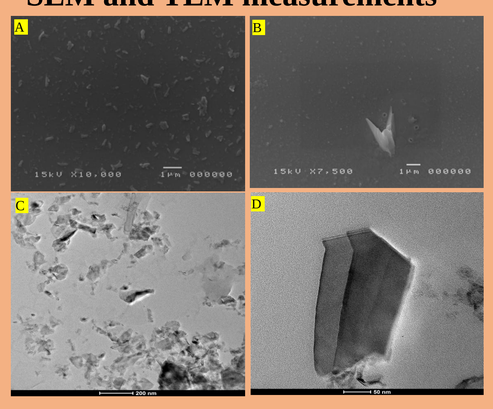
Material characterization

UV-Visible and Raman Spectroscopy measurements



- **(A-C) Shows the UV-Visible spectra of MoS_2 nanosheets centrifuged at 1000g and 2000g, stability profile of the same for one month; (D) shows the UV-Visible spectra of graphene oxide nanosheets; (E-F) shows the Raman spectra of graphene oxide and MoS_2 nanosheets respectively.**
- **MoS_2 exhibits 2D exciton parameters at 664 nm, 609 nm and 347 nm and 230 nm absorbance for GO NSs.**
- **Raman spectra shows peak shift with a wavenumber difference in the range of 23 cm-1 to 25 cm-1 resulting in few layer dispersion**

SEM and TEM measurements



(A-B) Shows the SEM measurement of MoS_2 nanosheets homogenously distributed over the substrate and sharp-edged structure; (C-D) shows the TEM measurement of water dispersed MoS_2 nanosheets showing sharp knife-like morphology.

Conclusion and Future studies

- **We have reported a significant improvement in the fabrication of MoS2 NSs by achieving a considerable amount of stability and concentration in pure water as a solvent.**
- **Apart from MoS_2, fabrication of GO in pure water with a very high initial concentration (600 and 1400 mg/mL) and thickness in the range of 1.2 nm - 2.5 nm has been achieved.**
- **MoS_2 showed a considerable bactericide effect in a short incubation time, 3–6 h, with both S. aureus and E. coli, whereas for GO the antibacterial action was lower and only began after 20 h incubation.**
- **GO showed completely different results exhibiting its antibacterial action after 20 h of incubation which we have ascribed to the so called 'wrapping mechanism,' due to large aggregates of GO NSs formed because of to the presence of different electrolytes in the given broth.**
- **MoS_2 only induced some antiviral action in virus the pre-treatment experiment. No antiviral effect was noted in either cell pre- and post-treatment case for both nanomaterials.**
- **The very interesting GO co-treatment case has puzzled the scenario because direct interaction of GO with virus is strong: we interpret this as due to the presence of specific glycoproteins on the Vero cell membrane that have high affinity with the oxygen functionalized groups on the GO NSs surfaces, such as carboxyl and epoxy.**

Our findings open very interesting prospects both :

(i) to understand the role of specific broth constituents and their chemical properties in view of GO and MoS2 NSs functionalization, when interacting with bacteria and viruses, and

(ii) also, exciting perspectives of applications given the specific antibacterial and antiviral observed actions.

(iii) In forthcoming experiments, we aim at studying also how the interactions of 2D NSs impact on genetic sequences of interacting viruses, to possibly unveil some of the interaction pathways.

References

1. Akhavan, O., Ghaderi, E., and Esfandiar, A. (2011). Wrapping bacteria by graphene nanosheets for isolation from environment, reactivation by sonication, and inactivation by near-infrared irradiation.J. Phys. Chem. B 115, 6279–6288. doi: 10.1021/jp200686k

2. Chen, Y. N., Hsueh, Y. H., Hsieh, C. T., Tzou, D. Y., and Chang, P. L. (2016). Antiviral activity of graphene–silver nanocomposites against non-enveloped and enveloped viruses. Intern. J. Environ. Res. Public Health 13:430. doi: 10. 3390/ijerph13040430

3. Hu, X., Yu, Y., Hou, W., Zhou, J., and Song, L. (2013). Effects of particle size and pH value on the hydrophilicity of graphene oxide. Appl. Surf. Sci. 273, 118–121. doi: 10.1016/j.apsusc.2013.01.201

Main research article

Our group's website- Nano-phor