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COMMUNICATION

Nitrate uptake by *p*-phosphonic acid or *p*-(trimethylammonium)methyl calix[8]arene stabilized laminar materials

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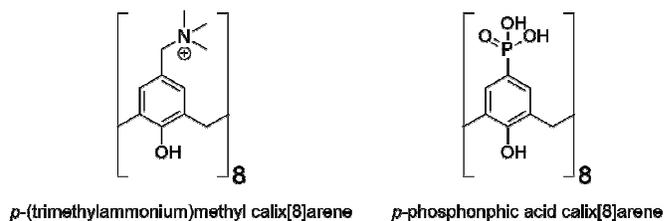
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Graphite, BN, MoS₂ and WS₂ are readily exfoliated using probe sonication in the presence of *p*-(trimethylammonium)methyl-calix[8]arene resulting in few-layer laminar material. The positively charged material is effective in the removal of nitrate ions from waste effluent, as is the negatively charged corresponding material stabilised by partially deprotonated *p*-phosphonic acid calix[8]arene, with scope for efficient recycling.

Two-dimensional (2D) materials such as graphene, hexagonal boron nitride (BN), molybdenum disulfide (MoS₂) and tungsten disulfide (WS₂) are gaining attention in biotechnology.¹⁻⁴ This is largely due to the development of improved methods of producing single sheets of these materials, allowing their novel properties to be elucidated and exploited. One such method uses sonic energy in the presence of *p*-phosphonic acid calix[8]arene to exfoliate and stabilise the 2D materials in aqueous solutions.⁵ The resultant single- and few-layer 2D materials are stable for months over a range of pH values, taking on negative charge associated with partial deprotonation of the upper rim phosphonic acid groups. Hereafter, in making reference to these calixarenes, referring to them as the phosphonic acid species, it is implied that they will be partially deprotonated. We note that the *p*-phosphonic acid calix[8]arene and the family of such calix[n]arenes, *n* = 4, 5, 6 and 8, are non-toxic,⁶ with demonstrated potential in drug delivery applications.⁷⁻⁹ This is an important factor for any applications, both for environmental and health concerns.



Another advantage to this exfoliation method is that the *p*-phosphonic acid calix[8]arene is non-covalently bound to the 2D material, limiting potential defects arising from covalent bonding to graphene for solubilisation purposes, while providing points of nucleation and growth of nanoparticles such as palladium, platinum and ruthenium into ordered arrays.¹⁰⁻¹² Of interest to us is the ability of graphene exfoliated and stabilised by the same calixarene using this methodology⁵ to absorb nitrate ions from waste water.¹³ In general, 2D materials are optimum candidates as molecular absorbents given that the exposed surface area of the materials is at the highest theoretically limit.

Nitrate removal from waste water is an important aspect of water treatment due to its hazardous effects on the environment and human health.^{14, 15} Excess nitrate can result in the formation of toxic algal blooms, in disrupting an already strained environment.^{16, 17} What is of particular interest is how the charge of the calixarene affects the exfoliation and stabilisation of 2D materials, as well as the ability of the exfoliated 2D material to remove nitrate ions from waste effluent. Herein we demonstrate that a positively charged *p*-(trimethylammonium)methyl-calix[8]arene can also facilitate the exfoliation of graphite, BN, MoS₂ and WS₂ and that the resulting suspension of the

composite material, along with the previously synthesised negatively charged *p*-phosphonic acid calix[8]arene exfoliated materials,⁵ can be effectively used to remove nitrate from waste effluent.

Fabrication and Characterisation of the 2D Materials

p-Phosphonic acid calix[8]arene was synthesised according to literature procedures.¹⁸ *p*-(Trimethylammonium)methyl-calix[8]arene was synthesised by mixing calix[8]arene, formaldehyde, acetic acid and dimethylamine in dimethylformamide at room temperature for 24 hours to afford *p*-(dimethylamine)methyl-calix[8]arene followed by mixing with iodomethane in dimethylformamide at room temperature for 4 hours (ESI).

The exfoliation method used in the present study has been described previously.^{5, 13} In the general experiment the 2D material (2 mg/mL) and the calix[8]arene (10 mg/mL *p*-phosphonic acid calix[8]arene or *p*-(trimethylammonium)methyl-calix[8]arene) was dispersed in Milli-Q water. The dispersion was then sonicated for 2 hours using a probe sonicator (150W at 70% amplitude, Sonifier cell disruptor, Model SLPt, Branson Ultrasonics Corporation). The dispersion was centrifuged at 1500 \times *g* for 30 minutes and the liquid removed, with the resulting solid redispersed in Milli-Q water (10 mL) and separated equally into six centrifuge tubes. These were centrifuged at 18400 \times *g* for 30 minutes and the liquid removed with the solid redispersed in the same volume of Milli-Q water. This last step was repeated 5 times on the six tubes to remove excess calix[8]arene. The dry masses in each tube tended to be very small and hence the weights had significant errors associated with them. For this reason all the measurements were compared as a process and not to a mass. The resulting dispersions are shown in Figure 1.

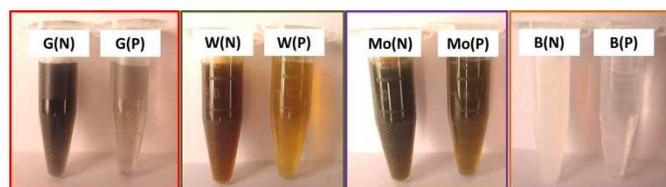
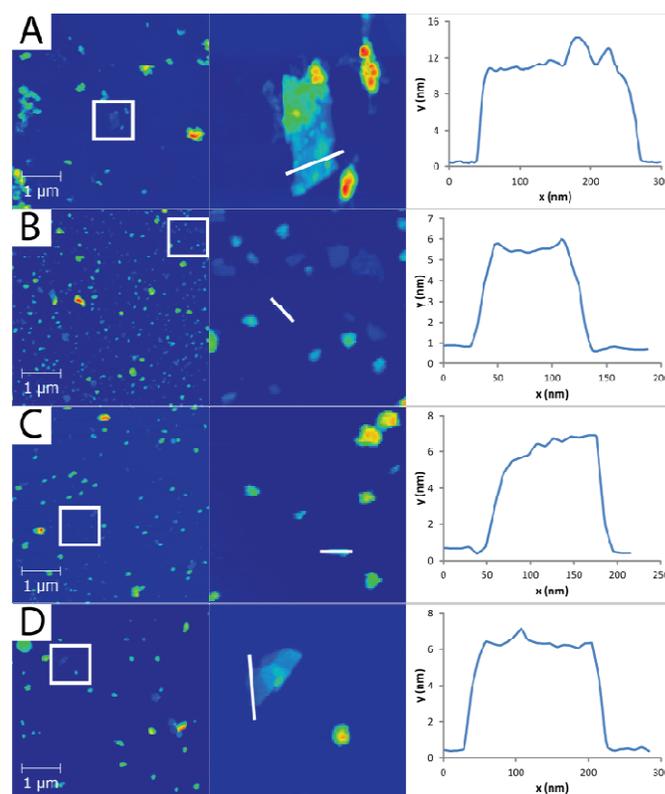


Figure 1. Dispersions of the 2D exfoliated materials graphene (G), WS₂ (W), MoS₂ (Mo), and BN (B) exfoliated in the presence of either *p*-(trimethylammonium)methyl-calix[8]arene (N) or *p*-phosphonic acid calix[8]arene (P).

The purification of the material by centrifugation partitions the different thickness of the laminar material with the initial low speed centrifugation removing the largely un-exfoliated material. At the other extreme, the high speed centrifugation partitions the calixarene and the highly aqueous stabilised single-layer material in supernatant from the few-layer and lower aqueous stabilised single sheet material in the pellet. This is evident from the initial high speed centrifugation, with the supernatant retaining the colour of the exfoliated 2D material. Some colour remains in the supernatant after each subsequent centrifugation washing step, with the intensity decreasing approximately exponentially through each subsequent centrifugation until it is difficult to discern after the final centrifugation. As a result the finally isolated exfoliated

2D materials for both the *p*-phosphonic acid and *p*-(trimethylammonium)methyl-calix[8]arenes are expected to be at most a few layers with a low concentration of single-layer sheets.

Exfoliation in the presence of *p*-phosphonic acid calix[8]arene has been described in a previous report,⁵ with the AFM images establishing the thickness of graphene, BN, MoS₂ and WS₂ at 2 nm, 4 nm, 5 nm and 5 nm respectively. The exfoliation method described herein increases the concentration of calixarene used from the original method, but produces a similar thickness of materials for both the *p*-phosphonic acid calix[8]arene (ESI) and the *p*-(trimethylammonium)methyl-calix[8]arene (Figure 2). The presence of *p*-(trimethylammonium)methyl-calix[8]arene appears to result in a greater exfoliation efficiency than in the presence of *p*-phosphonic acid calix[8]arene under identical exfoliation conditions. Qualitative evidence supporting this are the images of the suspended material in Figure 1, where the optical density of the *p*-(trimethylammonium)methyl-calix[8]arene exfoliated materials are higher than the *p*-phosphonic acid calix[8]arene exfoliated materials. It should be noted that the experiments in the present study were designed around identical exfoliation procedures to analyse the nitrate uptake of the various materials for exfoliation agents of different charge. Single-layer sheets are not necessarily ideal in this case. As noted in the purification method the single-layer sheets are likely to be extremely stable



in aqueous solution and require more time and energy to separate from the excess calix[8]arene.

Figure 2. Atomic force microscopy (AFM) images with 1 μ m \times 1 μ m inset and line profile of 2D materials exfoliated with *p*-(trimethylammonium)methyl calix[8]arene. A) Graphene, B) MoS₂, C) WS₂ and D) BN.

The transmission electron microscopy (TEM) images (Figure 3) show a significant number of *p*-(trimethylammonium)methyl-calix[8]arene exfoliated sheets from all 2D materials prepared. As in our previous report describing *p*-phosphonic acid calix[8]arene exfoliated materials,⁵ the TEM images in Figure 3a, 3b and 3c show reduced widths of the sheets relative to the widths of the starting material, graphite, MoS₂ and WS₂ flakes, suggesting that the materials were fragmented in the exfoliation process. The fragmentation presumably arises from the high energy cavitation associated with sonication. The BN sheets do not appear to have undergone significant fragmentation, with the 2D material still retaining its original circular shape. As noted earlier, the lack of single-layer sheets observed in the AFM images may be due to the purification process. However, the electron diffraction and high resolution TEM images for all of the materials (Figure 3) indicate that the exfoliated materials have a high degree of crystallinity. Thus, from the TEM and AFM images, it can be concluded that *p*-(trimethylammonium)methyl-calix[8]arene was effective in facilitating the exfoliation and stabilisation of graphene, BN, MoS₂ and WS₂ in water.

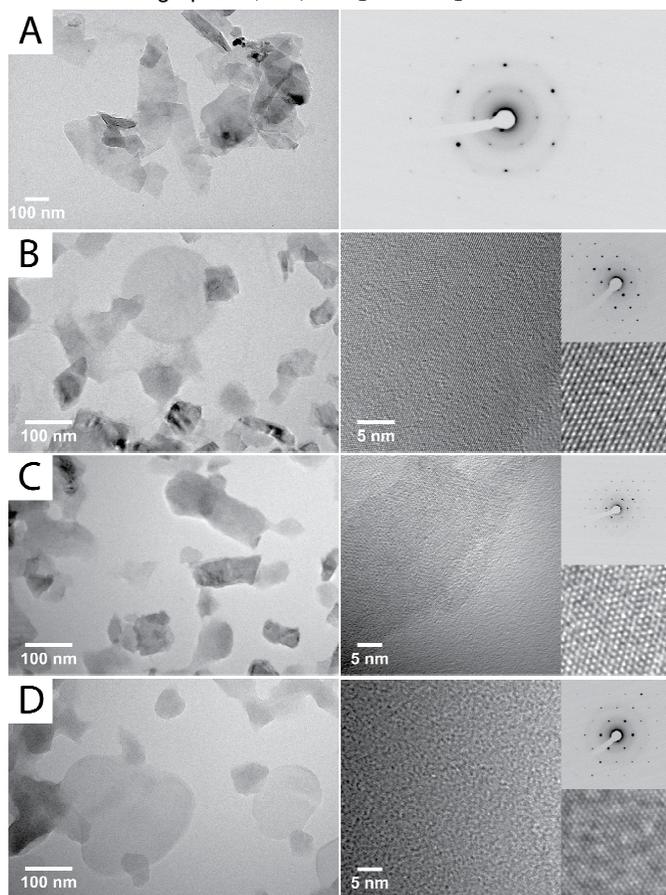


Figure 3. TEM images with 5 nm x 5 nm inset and electron diffraction of 2D materials exfoliated with *p*-(trimethylammonium)methyl-calix[8]arene. A) Graphene, B) MoS₂, C) WS₂ and D) BN.

Nitrate Removal

Removal of nitrate ions from effluent is legislated in many countries. According to the United States Environmental

Protection Agency (EPA), the maximum level of nitrate-nitrogen [NO₃⁻-N] in drinking water is regulated so that it is below 10 mg/L (equivalent to approximately 45 mg/L as NO₃⁻).^{14, 19} Nitrate-nitrogen [NO₃⁻-N] represents the amount of nitrogen (N) present in aqueous solutions yielding from nitrate ions (NO₃⁻).¹⁹ In the present study, nitrate uptake for the final mixtures of various 2D materials stabilised by *p*-phosphonic acid calix[8]arene or *p*-(trimethylammonium)methyl-calix[8]arene was assessed. The final mixtures were diluted three times with Milli-Q water, centrifuged at 18400 x *g* for 30 minutes and the majority of supernatant discarded. The remaining mixtures (0.25 mL) were then diluted with nitrate-containing aquatic effluent which is also composed of phosphates, carbonate buffer, vitamins, and some micronutrients (1.25 mL, pH 7.5).²⁰ The concentration of nitrate in the effluent was monitored at various time intervals (0, 3, 6, 12, 18 and 24 hours). Prior to nitrate analysis, each mixture was centrifuged at 18400 x *g* for 30 minutes and the suspension-free supernatants were collected for spectrophotometric analysis.²¹ The reusability of the materials for nitrate removal was tested after initially exposing the 2D materials to the effluent for 24 hours. The samples were then centrifuged at 18400 x *g* for 30 minutes, the supernatant discarded, the solid re-dispersed in Milli-Q water and subjected to mild sonication (180 W) for 2.5 minutes.¹³ These samples were then re-centrifuged at 18400 x *g* for 30 minutes, the major part of the supernatant was then discarded (1.25 mL), and replaced with same amount of nitrate-containing aqueous media. This second cycle was analyzed after exposure to the effluent for 24 hours following the same procedure as the first cycle.

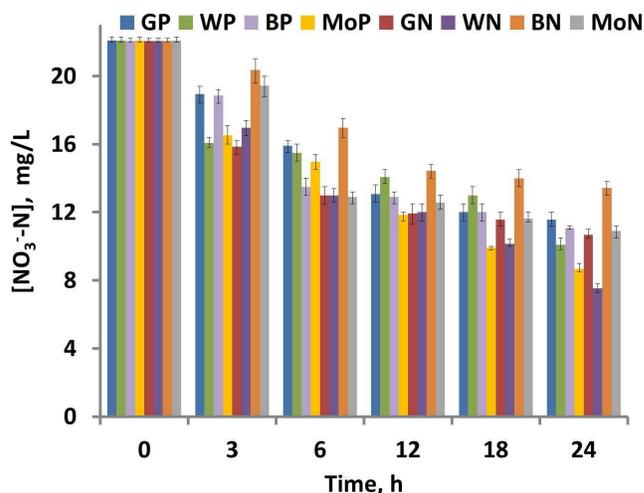


Figure 4. Nitrate-nitrogen [NO₃⁻-N] content of liquid solution, exposed to various exfoliated laminar materials stabilised by *p*-phosphonic acid calix[8]arene or *p*-(trimethylammonium)methyl-calix[8]arene, designated "P" and "N" respectively with G = Graphene, W = WS₂, B = BN, Mo = MoS₂.

For all eight of the laminar materials produced, regardless of which exfoliating compound was used, we observed a substantial amount of nitrate removal from the effluent media (Figure 4). The WS₂ sample exfoliated with *p*-(trimethylammonium)methyl-calix[8]arene (WN) had the highest removal at 66 %, and removal by graphene and WS₂ exfoliated

with *p*-(trimethylammonium)methyl-calix[8]arene were slightly more efficient than their *p*-phosphonic acid calix[8]arene exfoliated counterparts. Generally, the laminar materials exfoliated with *p*-(trimethylammonium)methyl-calix[8]arene reached higher nitrate removal rates within the first six hours of the experiment relative to *p*-phosphonic acid calix[8]arene exfoliated materials, with the exception of BN. The maximum nitrate removal efficiencies were consistent with our previous study of *p*-phosphonic acid calix[8]arene exfoliated graphene sheets reaching a maximum removal efficiency of 55.7% for different exfoliation conditions.¹³

The variations within the nitrate uptake are presumably related to the amount of exfoliated surfaces available, the functional groups of the calix[8]arenes and/or the manner in which the calix[8]arenes adsorb to the surface. As indicated by Figure 1 and 5, the *p*-(trimethylammonium)methyl-calix[8]arene resulted in apparently greater exfoliation. Thus, given the difference in amount of material exfoliated and the differences in nitrate removal efficiencies, it would appear that the phosphonic acid calix[8]arene has a greater efficiency at nitrate removal per mass. This establishes that the negatively charged *p*-phosphonated calixarene is more effective at binding nitrate than the more electrostatically favoured trimethylammonium cation functionalised calixarene. However, the *p*-(trimethylammonium)methyl-calix[8]arene with WS₂ is the best material for nitrate removal as it yielded the lowest [NO₃⁻-N] concentration (7.5 mg/L) in the remaining effluent, under the same preparation procedures as for all other materials.

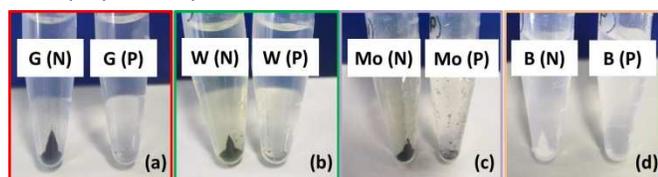


Figure 5. Images of graphene (G), WS₂ (W), BN (B), MoS₂ (Mo) exfoliated by either *p*-phosphonated calix[8]arene (P) (G(P); W(P); B(P); Mo(P)) or *p*-(trimethylammonium)methyl-calix[8]arene (N) (G(N); W(N); B(N); Mo(N)) in a mixture with nitrate containing aqueous solution, followed by their centrifugation at 18400 x *g* for 30 minutes.

Nitrate removal efficiencies of these laminar materials after two consecutive cycles were also compared (Table 1). The recycling process was applied to the samples at a time-interval of 24 hours. These results indicate that all laminar materials were still effective for removing nitrate during their second cycle (Table 1), despite having a decrease in their removal efficiencies. Independent from its surface group, graphene was more stable in recycling than the other laminar materials, showing similar nitrate removal efficiencies for each consecutive cycle. In terms of overall nitrate removal efficiencies of two-consecutive cycles, MoP and WN gave the highest removal efficiencies of 53.2% and 51.8%, respectively.

Table 1. Nitrate-nitrogen [NO₃⁻-N] removal efficiencies of various laminar materials in two consecutive cycles using the same materials. The values represent the amount of Nitrate-nitrogen [NO₃⁻-N] ions removed from solution (See Figure 5 for abbreviations).

| Sample | GP | GN | WP | WN | BP | BN | MoP | MoN |
|------------------------------|------|------|------|------|------|------|------|------|
| 1 st cycle (mg/L) | 10.6 | 11.0 | 11.5 | 14.0 | 11.5 | 9.0 | 13.5 | 10.5 |
| 2 nd cycle (mg/L) | 9.5 | 8.5 | 6.5 | 8.8 | 4.9 | 6.6 | 9.9 | 8.0 |
| Total (mg/L) | 20.1 | 19.5 | 18.0 | 22.8 | 16.4 | 15.6 | 23.4 | 18.5 |
| Overall Efficiency (%) | 45.7 | 44.3 | 40.9 | 51.8 | 37.3 | 35.5 | 53.2 | 42.0 |

Of interest in an industrial sense is that although these calixarene-exfoliated materials are stable for months prior to the addition of effluent, once the effluent is added the material begins to precipitate. We hypothesize that this may be the result of the nitrate ion acting as an intercalation agent between exfoliated sheets, creating a weak glue to bind the laminar sheets. Indeed, this precipitation of environmentally benign materials from effluent may be a viable method for lowering [NO₃⁻-N] content to below regulated concentrations.

Conclusions

We have demonstrated the exfoliation of 2D materials from bulk materials with a positively charged calix[8]arene (*p*-(trimethylammonium)methyl-calix[8]arene). The synthesis is a simple two-step process from a known high yielding preparation of calix[8]arene. We have shown that laminar materials exfoliated with either positively charged or negatively charged calix[8]arenes were effective at removing nitrate from waste effluent, with WS₂ exfoliated in the presence of *p*-(trimethylammonium)methyl-calix[8]arene resulting in the highest decrease in nitrate content of the waste effluent. All materials show significant recyclability removing only slightly less nitrate after regeneration by mild sonication. One benefit of these abundant laminar earth minerals is they begin to precipitate after binding nitrate allowing a facile method of nitrate extraction.

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Notes and references

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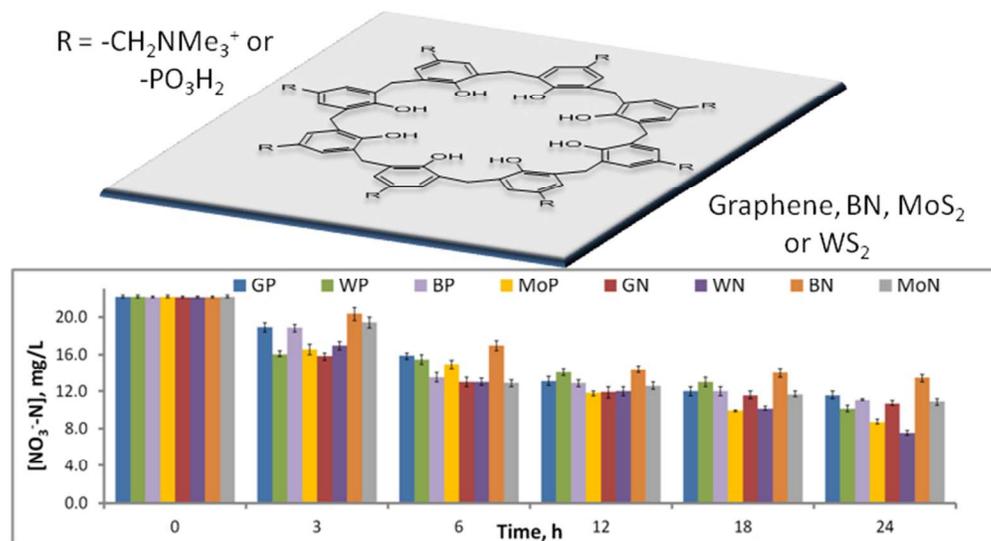
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Electronic Supplementary Information (ESI) available: AFM images of Graphene, MoS₂, WS₂ and BN exfoliated with *p*-phosphonic acid

calix[8]arene and the synthetic procedure for *p*-(trimethylammonium)methyl-calix[8]arene. See DOI: 10.1039/c000000x/

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Graphite, BN, MoS₂ and WS₂ are exfoliated and stabilised in water with positively or negatively charged non-toxic calix[8]arenes. All 2D materials adsorb nitrate from waste effluent, precipitate once nitrate is bound and are regenerated.