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# An Improved Method to Wash Graphene Prior to Use as a Drug Delivery Vehicle

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## Abstract

Due to its ultra-high surface area and ability to be functionalized, graphene is suitable for use in many biomedical applications, including gene and drug delivery. After the manufacturing process of graphene oxide (GO), washing steps are necessary for neutralization and removal of impurities. By using a centrifuge, these washing steps are laborious and time-consuming. An improved method for washing GO dispersions to rapidly neutralize pH levels is described using tangential flow filtration (TFF) with the Vivaflow® 50, 100 kDa MWCO. TFF shows, in comparison to conventional centrifugation, a significant reduction in time needed with high recoveries of GO.

## Introduction

Graphene is a one-atom-thick carbon allotrope in a hexagonal lattice formation (2D monolayer) that is isolated from graphite crystals.[1,2] This substance has been investigated for use in many biomedical applications, including gene and drug delivery,[1,3] photo thermal therapy[4] and cellular imaging.[5] The unique surface of graphene, which contains delocalized electrons in an ultra-high surface area,[1] allows for the efficient loading of hydrophobic drugs by π-π interactions.[3,6]

One of the most used types of graphene is GO, which is produced via Hummers' method by chemically exfoliating graphite into individual sheets through oxidation. However, lengthy treatments are required after manufacturing to remove contaminants, including potassium and sulfide impurities, and neutralize the high acidity of the sheets. This process typically uses multiple centrifugation steps that vary between 4 and 10 hours each. It is not uncommon for the complete process to take up to one week in total,[2,7,8] with centrifuge capacity also being a limiting factor for larger batch sizes. For biomedical applications, regulating the pH of graphene oxide is of the essence for compatibility with biological systems. pH also plays an important role in drug release, due to graphene oxide's pH-dependent amphiphilicity.[9,10]

In this study, we aimed to develop an improved, more efficient process for the removal of contaminants and pH adjustment for GO. Robust, lab-scale tangential flow filtration (TFF) with Vivaflow® offered an economical solution to enable significantly faster, convenient processing (Figure 1).

## Materials and Methods

The tangential flow in crossflow systems such as Vivaflow® helps to minimize membrane fouling and enables the processing of larger batch volumes. The separate sample reservoir offered as part of the Vivaflow® system enables simple diafiltration while maintaining a constant sample volume and can easily be placed under sonication to prevent aggregation of graphene sheets—a feature of this nanomaterial that can impair effective washing and pH adjustment. Initial experiments with Vivaspin® 6 centrifugal concentrators showed that PES membranes were rapidly fouled by graphene (data not shown). We therefore selected Vivaflow® 50 with a 100 kDa MWCO regenerated cellulose (RC) membrane for our comparative study with the centrifugation method.

**Method Comparison of Centrifugation and TFF (1st Experiment)**

To adjust the pH of GO dispersion by centrifugation, 50 mL centrifuge tubes were filled with 100 µg/mL graphene oxide, mildly sonicated for 10 mins (in a sonic bath) and then centrifuged at 4,696 *g* for 1 or 10 h. The supernatant was decanted, and the graphene pellet resuspended with ultrapure water (Arium®). Measurements of pH and concentration (using UV-vis spectrometry at a wavelength of 660 nm) were taken and the process was repeated seven times, including one wash (the fifth) with 50% v/v ethanol.

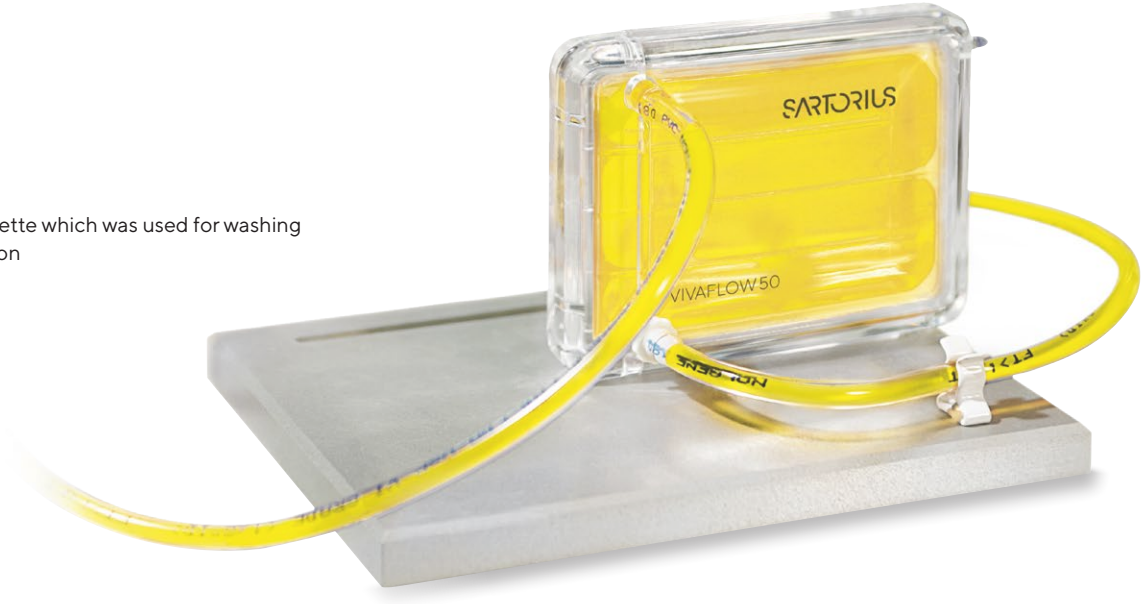


Figure 1: Vivaflow® TFF Cassette which was used for washing of Graphene Oxide dispersion

To adjust the pH of graphene oxide by TFF using Vivaflow®, a relative volume of 400 mL graphene oxide (equivalent to 8 x 50 mL tubes using the centrifugation method) at 100 µg/mL was added to a Vivaflow® sample and diafiltration reservoir, which was kept under mild sonication (in a sonic bath). GO sheets were washed by diafiltration with ultrapure water (Arium®) using a Vivaflow® 50 cassette with 100 kDa MWCO RC membrane, operated at 2.5 bar pressure (measured at the outlet). After each liter of diafiltration (2.5 x exchange volumes), 10 mL samples were taken to measure the pH and concentration (using UV-vis absorbance)—these samples were returned to the sample reservoir for subsequent diafiltration steps. In total, 7 x 1 liter exchange solvent was used to wash the graphene—all steps using water, except the fifth, which used 50% v/v ethanol.

### Washing of Graphene Oxide by TFF Using Vivaflow® (2nd Experiment)

To confirm repeatability, a GO dispersion with a concentration of 100 µg/mL was prepared. The pH was measured to be 3.5. Each of these washing experiments, conducted in duplicate, used 250 mL of graphene oxide dispersion. A sample of 10 mL was taken from the reservoir after 1 liter exchange of Arium® ultrapure water (4 exchange volumes) and absorbance, for determination of graphene oxide concentration, and pH were measured. A further sample of 10 mL was taken at the end of the filtration process. This included another liter of exchange solution (a total of 8 exchange volumes), constituted of 250 mL of 50% (v/v) ethanol and 750 mL of Arium® ultrapure water. Absorbance and pH of the sample were measured.

## Results

In the first experiment (Figure 2), three methods for washing 400 mL of GO (100 µg/mL) were compared in terms of time required to reach neutral pH and GO recovery: 1) centrifugation with 1-hour spins (short); 2) centrifugation with 10-hour spins (long); and 3) TFF. Using 1-hour centrifugation steps, the pH of the starting GO material was adjusted from approximately 4.5 to 6. However, short spins such as this have a severely detrimental impact on the recovery of graphene, due to significant losses in the supernatants—particularly of the smaller sheets, which are the most useful for drug delivery applications. Recovery was determined to be around 16% of the total starting material. Increasing the length of each centrifugation step to 10 hours dramatically improved recovery (93%) but with the effect being an undesirable process time of approximately 200 hours. Vivaflow® enabled neutralization of graphene oxide pH in 10.5 hours, with each diafiltration step taking approximately 90 minutes. GO recovery was determined at 97%.

In the second experiment (Figure 3), 250 mL of GO with a concentration of 100 µg/mL was washed using TFF. pH and GO recovery were measured at the beginning and after 4 and 8 exchange volumes of the wash solution to map the progression of the process. After washing with 8 exchange volumes of the initial sample volume, i.e. 2 liters, a pH of 6.9 could be achieved. The average recovery of GO was determined to be 81%. This slightly lower recovery than in the first experiment, beside experimental variations, can be explained by the lower initial sample volume, where the effects of fouling | adsorption become more apparent.

**Washing of Graphene Oxide Centrifugation and TFF in Comparison by Recovery and Time**

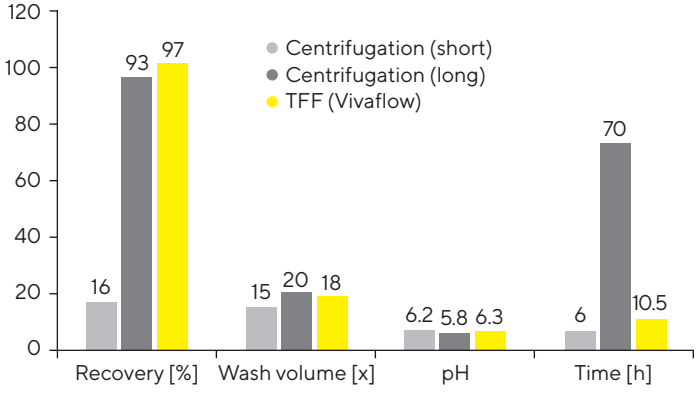


Figure 2: Comparison of methods for washing a graphene oxide dispersion (400 mL, 100 µg/mL) with respect to the recovery of graphene oxide after the washing process and the total time required to reach a neutral pH (n = 1). Centrifugation (short) = 7 cycles with 1-hour steps each, Centrifugation (long) = 7 cycles with 10-hour steps each and TFF (Vivaflow®) by continuous buffer exchange.

**Washing of Graphene Oxide by TFF (Vivaflow): Recovery and pH**

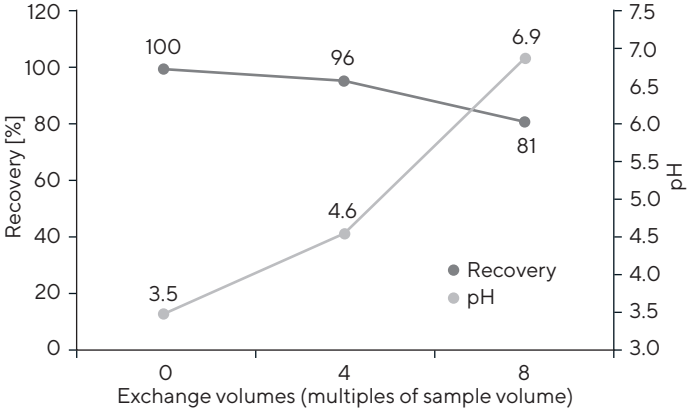


Figure 3: Washing of a graphene oxide dispersion (100 µg/mL) using a Vivaflow® TFF cassette (RC membrane) with a MWCO of 100 kDa to reach a neutral pH. Recovery of graphene oxide and the pH of the dispersion are shown as a function of the exchange volume of the wash solution as a multiple of the feed volume of 250 mL (n = 2).

## Conclusions

Three methods of washing to neutralize graphene oxide—centrifugation with short and long steps, and TFF using Vivaflow® (100 kDa MWCO RC)—were compared and the time course of TFF in terms of pH and recovery was plotted. Using TFF where the removal of the original solvent is through an ultrafiltration membrane, there was effectively no loss of the biomedically relevant, smaller graphene sheets when using Vivaflow®. Recoveries of graphene oxide were high and at least comparable to, if not higher than, traditional centrifugation approaches. Vivaflow® also dramatically reduced processing time for the removal of contaminants and pH neutralization. Total handling time of 189.5 hours (95%) or 59.5 hours (85%) of instrument-only time was saved by the improved method. Furthermore, TFF reduces the need for the special handling of large volumes of graphene oxide supernatant waste containing the smaller sheets, making it a more ecological process. Furthermore, since the washing process can be performed continuously when using TFF, labor is significantly reduced through the avoidance of sample manipulations that are inherently required with the conventional centrifugation approach.

TFF could represent an ideal solution to make the manufacture of graphene more commercially viable at large scales through the marked increase in process speed without sacrificing the yield of graphene oxide.

## Abbreviations

| | |
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| GO | Graphene oxide |
| TFF | Tangential flow filtration |
| MWCO | Molecular weight cut off |
| 2D | Two-dimensional |
| PES | Polyethersulfone |
| RC | Regenerated cellulose |
| UV-vis | Ultraviolet-visible |

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