Adsorption of antiretroviral drugs, efavirenz and nevirapine from aqueous solution by graphene wool: Kinetic, equilibrium, thermodynamic and computational studies

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ABSTRACT

The increasing concentrations of pharmaceutical and personal care products in water bodies have attracted attention due to the risk of non-target exposures. The application of any graphene-based material for the remediation of antiretroviral drug contamination has not been reported, therefore graphene wool was synthesized by chemical vapour deposition as adsorbent for the removal of efavirenz (EFV) and nevirapine (NVP) from water. Results revealed that adsorption of EFV was best fitted to the intraparticle diffusion model, with multilinearity (multiple adsorption steps). The pseudo-second-order model best describes GW-NVP interaction. Isotherm parameters revealed that Sips and Freundlich model best fit GW-EFV and GW-NVP interactions, with the least value of SSE < 0.04 and 1.27, respectively. GW demonstrated higher adsorption capacity and adsorption maxima for NVP with $K_d$ and $q_m$ values of $\sim 2.54 \text{ L/g}$ and $\sim 48.31 \text{ mg/g}$, compared to $\sim 1.48 \text{ L/g}$ and $\sim 4.41 \text{ mg/g}$ obtained for EFV adsorption. Isotherm parameters suggest that GW adsorbed NVP slightly better with stronger binding strength than EFV, with removal efficiencies of 84% (NVP) and 80% (EFV) under optimum conditions. A heterogeneous adsorption mechanism was suggested for GW-EFV sorption, in contrast to a less heterogeneous and multilayer adsorption mechanism for GW-NVP adsorption. NVP adsorption is a spontaneous exothermic process, while GW-EFV interaction is a spontaneous endothermic process. Experimental results were supported by computational studies, which revealed the influence of strong dispersion interactions and H-bonding at specific pH ranges.

1. Introduction

Pharmaceuticals and personal care products (PPCP) are a vast and unique class of emerging chemical pollutants (ECP). They may cause physiological effects and alter systemic processes in humans upon exposure to low concentrations, which makes non-target exposures worrisome [1, 2]. In the last decade, the occurrence of antiviral and antiretroviral drugs as microcontaminants in drinking and surface water has received significant attention in both developing and developed parts of the world [3-6]. The risk assessment and adverse effects of exposure to these chemicals are not fully understood, but there is growing scientific, public, and regulatory concern as these drug compounds have been detected in surface waters [7-9]. The toxicological profile of selected antiviral drugs suggests that they are hazardous to aquatic fauna, with a low maximal effective concentration (EC_{50}) value of 57 mg/L [4]. Several antiviral drugs and their metabolites are nonbiodegradable, hence they persist in the environment [6, 10].

Antiretroviral drugs (ARVDs) are therapeutic agents for the treatment of retroviral infections, primarily the human immunodeficiency virus type 1 (HIV-1). The virus that causes HIV disease attacks the CD4-T cells responsible for body immunity, thus making humans vulnerable or susceptible to infections and diseases [11-12]. Antiretroviral treatment therapy against HIV-1 does not eliminate the virus but inhibits its rapid replication and increases the life expectancy of infected people [13].

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Table 1
Physicochemical properties of target antiretroviral drugs (ARVD).

| ARVD (and 
<table>
<thead>
<tr>
<th>abbreviation)</th>
<th>Molecular</th>
<th>LogK&lt;sub&gt;ow&lt;/sub&gt;</th>
<th>S&lt;sub&gt;ow&lt;/sub&gt;</th>
<th>MM</th>
<th>pK&lt;sub&gt;a&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efavirenz (EFV)</td>
<td>C&lt;sub&gt;15&lt;/sub&gt;H&lt;sub&gt;24&lt;/sub&gt;ClF&lt;sub&gt;4&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>4.70</td>
<td>0.093</td>
<td>315.68</td>
<td>10.20/12.52</td>
</tr>
<tr>
<td>Nevirapine (NVP)</td>
<td>C&lt;sub&gt;14&lt;/sub&gt;H&lt;sub&gt;16&lt;/sub&gt;N&lt;sub&gt;4&lt;/sub&gt;O</td>
<td>3.89</td>
<td>0.705</td>
<td>266.30</td>
<td>2.80</td>
</tr>
</tbody>
</table>

Log K<sub>ow</sub>: octanol–water partition coefficient, S<sub>ow</sub>: water solubility (mg/L), MM: molar mass (g/mol), pK<sub>a</sub>: log of acid-dissociation constant. Cited from pubchem [25,26].

About 90% of orally administered drugs are removed unaltered or partially metabolized from the body as faecal waste, and they are thus found in sewage waste [14]. Similarly, unused and expired drugs may be disposed indiscriminately resulting in contamination of drainage systems and other water bodies [15].

Efavirenz and nevirapine exert distinct pathways for the inhibition of the proliferation of HIV, and they are classified amongst the most common ARVDs [16]. Adsorption of efavirenz onto biosolids has recently enhanced the removal efficiency of ARVDs in conventional wastewater treatment plants (WWTPs) in South Africa, with an efavirenz sludge concentration as high as 43 mg/kg, while 22.5% removal efficiency of nevirapine was also reported [17]. A Kenyan study reported reportedly enhanced the removal efficiency of ARVDs in conventional secondary pollutants or undesirable by-products [22-24].

Operational cost, easy adaptability, and minimal tendency of generating waste products has benefits such as simplicity in design and operation, low maintenance, and reduced carbon footprint [25,26].

Adsorption techniques arising from material science advances have proven useful in mitigating the challenge of emerging chemical pollutants (such as pharmaceuticals) in water [19-21]. The adsorption method for the decontamination of water polluted with organic chemical pollutants has benefits such as simplicity in design and operation, low operational cost, easy adaptability, and minimal tendency of generating secondary pollutants or undesirable by-products [22-24]. Graphene-based materials (GBMs) have been harnessed as efficient next-generation sorbents for water purification applications, because their surface is largely hydrophobic, porous, and possesses high adsorption affinities for a vast number of organic contaminants (OCs) [19,21,22]. However, the application of graphene-based materials for the remediation of any antiretroviral drug contamination in water has not been reported previously.

Therefore, the overall aim of this study was to synthesize graphene wool (GW) for the removal of selected ARVDs from an aqueous solution. Sorption isotherm, kinetics, thermodynamic and computational studies were carried out to elucidate and evaluate the sorption mechanism(s) of GW-ARVD interactions, as well as the adsorption capacities and removal efficiency of graphene wool for potential use in the purification of ARVD-contaminated water. Table 1 provides a summary of the basic physicochemical properties of the target ARVDs chosen for this study. Efavirenz and nevirapine were chosen as target antiretroviral drugs based on their toxicity, prevalence and persistence in surface waters [4].

2. Experimental methods

2.1. Synthesis of graphene wool (GW)

Graphene wool was synthesized using an established bottom-up approach as reported in [27]. Briefly, quartz wool (Arcos Organics, New Jersey, USA) was carefully arranged at the centre of a horizontal quartz tube (50 mm o.d., 44 mm i.d., x 1 m length) in a high-temperature furnace (OTF-1200X-50–5 L, MTI Corporation, California, USA). 500 sccm argon and hydrogen (analytical grade, 99.999%, Afrox, South Africa) were pre-mixed and released into the thermal reactor at 1200 °C. The quartz wool was annealed under this temperature and gas flow for 10 min, thereafter methane gas (analytical grade, 99.95%, Afrox, South Africa) was added for graphene growth. The system was cooled under Ar and H<sub>2</sub> gas after the optimized growth time had elapsed. Thus, the graphene wool (GW) synthesized is a composite of quartz wool coated with graphene.

2.2. Characterisation of graphene wool (GW) adsorbent

Surface morphology was examined using scanning electron microscopy (SEM) with aid of a Zeiss Ultra-Plus 55 field emission scanning electron microscope (FE-SEM), operated at 2.0 kV (OXFORD Link-ISIS-300 Zeiss, Germany); High-resolution transmission electron microscopy (TEM) images were taken using a JEOL JEM 2100F (JEOL Ltd, Tokyo, Japan) operated at 200 kV. The crystal structure of GW was also examined using a Bruker VD 2D Phaser X-ray diffraction (XRD) instrument with reflection geometry at 20 values (10 – 60°) with a 5.24 squisition time per step, operated with a Cu Kα radiation source (λ = 0.15406 nm) at 50 kV and 30 mA. The surface area and porosity of GW were determined by N<sub>2</sub> adsorption-desorption isotherms at 77 K, using a NOVA Touch Surface analyzer system (Anton Paar, South Africa) in a relative pressure (P/P<sub>0</sub>) range of 0.01–1.0, following a model of Brunauer–Emmett–Teller (BET) and Barrett–Joyner–Halenda (BJH) techniques.

2.3. Adsorption kinetics and isotherm experiments

Batch adsorption experiments of efavirenz (EFV) and nevirapine (EFV) onto GW adsorbent were carried out in 40 mL PTFE screw-capped vials (Stargate Scientific, South Africa) at 25 ± 1 °C. Background electrolyte contained 0.01 mol/L CaCl<sub>2</sub> (analytical grade, ACE, South Africa) in deionized water (9.2 μS/cm<sup>3</sup>, Millipore, Bedford, MA, USA), and 200 mg/L NaNO<sub>3</sub> (analytical grade, Sigma-Aldrich, Germany) to inhibit microbial activity. Kinetic experiments were conducted for 72 h with initial EFV and NVP concentrations of 5 mg/L (99% pure standards, analytical grade were purchased from Sigma-Aldrich, Germany), and the mass per volume ratio was 10 mg of graphene wool per 5 mL solution.

Afterwards, the isotherm experiments were carried out with initial concentrations of ARVD solutions ranging between 1 - 20 mg/L, respectively. Desorption experiments were carried out by adding fresh 5 mL of background electrolyte after the supernatant from the adsorption studies had been decanted. Sorption thermodynamic evaluations were carried out at varying temperatures of 298, 308, and 318 K using a thermostated shaking water bath (Wisebath, Celsius Scientific, South Africa). The role of solution pH was studied over the pH range of 2 - 13, and the pH of the solutions was adjusted using 0.1 M HCl (analytical grade, Merck, South Africa) and/or 0.01 M NaOH (analytical grade, ACE, South Africa). Adsorption performance of GW for the removal of EFV and NVP was also studied at varying contact times (0, 15, 30, 60, 120, 240, 360, 600, 840, 1440, 2160, 2880, 3600, and 4320 mins, respectively). All experiments were carried out in duplicate.

2.4. Quantification

After equilibration for 24 h, the vials were centrifuged at 3000 rpm for 5 min to obtain a clear supernatant, which was then filtered using a 0.22 μm syringe filter (Stargate scientific, South Africa) and collected in 2 mL LC vials for analysis by ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS).

2.4.1. UPLC-MS/MS analysis

Analysis of the supernatant was carried out using sensitive and rapid ultra-performance liquid chromatography (UPLC); accompanied by
Electrospray ionization (ESI) in positive and negative mode (NVP and EFV, respectively) and mass spectrometric (MS) detection with a triple-quadrupole MS/MS system (Waters Inc., Milford, Massachusetts, USA). Chromatographic separation was achieved using an Acquity UPLC® ethylene bridged hybrid (BEH) shield reversed-phase C18 column (1.7 μm particle size, 100 mm length × 2.1 mm internal diameter) equipped with an Acquity UPLC C18 guard column (Waters, Milford, MA). EFV and NVP separation was carried out with 0.1% formic acid (HPLC grade) in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B, HPLC grade), while the temperature of the column was maintained at 40 °C, the flow rate was set at 0.3 mL/min and a gradient run time of 5 min. The LC system was equipped with an autosampler with 5 μL injection volume per analysis, and samples were analysed in duplicate (n = 2) and standards in triplicates (n = 3). The MS discharge electrode was set at 5 μA for electrospray negative and positive ionization for EFV and NVP, respectively; the gas temperature was set at 250 °C, the sheath gas temperature at 300 °C, the gas flow rate was set at 10 L/min, Delta EMV set at 400 V, nebuliser pressure of 35 psi, and capillary voltage was set at 3000 V.

Data acquisition and analysis was carried out using MassLynx™ (version 4.2) software (Waters, Milford, MA) and QuanLynx Method Editor V4.2 was used to prepare the calibration curve and quantification of EFV and NVP. The calibration curves were obtained from stock solutions of each ARVD with regression coefficient (R²) > 0.98. The working standards were in the range of 0.01 – 5 mg/L for EFV and NVP, respectively. Equilibrium concentrations (Cₑ) were deduced from the equation of the curve and the amount adsorbed was calculated using the mass-balance equation presented below:

\[ q_e = \left( \frac{C_0 - C_e}{S_m} \right) \]

Where C₀ (mg/L) is the initial concentration, Cₑ (mg/L) is the equilibrium solute concentration, V₀ is the initial volume (L) and Sₘ is the mass (g) of the adsorbent.

Removal efficiency (%) = \frac{(C_0 - C_e)}{C_0} \times 100

2.5. Computational study of GW-ARVD interaction

EFV and NVP, in various conformers and tautomers, were optimized with Density Functional Theory (DFT), using B3LYP/6–31G(d) and the conductor-like polarizable continuum solvation model (CPCM) with water. Interactions between EFV, NVP, and various forms of a 5 × 5 graphene sheet (including functionalization with –O and –OH groups) were optimized. All structures are true local energy minima unless otherwise noted. All electronic structure calculations were performed with Gaussian 16, rev. C [28]. Selected wavefunctions were further investigated with the Fragment, Atomic, Localized, Delocalized and Interatomic (FALDI) density decomposition scheme [29, 30] using in-house codes and in conjunction with atomic basins defined by the Quantum Theory of Atoms in Molecules (QTAIM) [31], as implemented.
in AIMAll v. 19.10.12 [32]. FALDI was used to calculate and visualize inter-fragment delocalization indices (DIs), through the joint overlap of atomic overlap matrices. FALDI delocalization indices (DIs) were performed without the localized-delocalized overlap correction [30] and correspond to orthodox QTAIM-defined DIs. Visualizations of FALDI fields were performed with visual molecular dynamics (VMD) [33].

3. Results and discussion

3.1. Characterisation of graphene wool

Figure (1a) reveals that graphene wool (GW) has an amorphous structure with an intercellular spacing of $d_{002} = 3.70 \, \text{Å}$, confirmed by the broad diffraction plane C (002) appearing at $2\theta = 22.5^\circ$. The shift of carbon plane (002) to lower Bragg angle (26.4$^\circ$ to 22.5$^\circ$) and broad peak confirms the coverage of the wool substrate by several layers of graphene, thus creating an amorphous carbon phase [34]. The hump nature of the diffraction pattern is a result of the presence of amorphous silica substrate with weak peaks at 30$^\circ$ and 42$^\circ$ respectively [35, 36]. The XPS and Raman spectra (Fig. 1b & 1e) revealed sp2 $C = C$ structure as the most intense peak at 284.6 eV, which reflects a partially disordered network of the graphene multilayer structure and accounts for the D peak in the Raman spectrum (Fig. 1f) [27, 37–39].

The SEM and TEM analysis provided high-resolution microscopic images of GW (Fig. 1c & 1d). The analysis revealed that the diameter of each strand of GW is between 6 - 8 μm and extensive coverage of the substrate by graphene, with a rough and heterogeneous surface morphology. The BET analysis revealed a H4-isotherm curve associated with complex materials/composites (Fig. 1f) and the pore size distribution plot (Fig. 1g) showed that GW adsorbent has both micropores (pore diameter $> 2$ nm) and mesopores (2 nm $< $ pore diameter $< 50$ nm), but no macropores [39]. The specific surface area (SSA), pore volume, and pore diameter were 29.6 m$^2$/g, 0.039 cc/g, and 1.37 nm respectively.

3.2. Adsorption kinetics and effect of contact time

The pseudo-first-order (PFO) eq. (3), pseudo-second-order (PSO) eq. (4), and Weber-Morris intraparticle diffusion eq. (5) models were used to fit the time-concentration profile of adsorption of efavirenz and nevirapine onto synthesized graphene wool adsorbent (Fig. 1). Reaction pathways in adsorption processes are influenced by contact time. The sorption rate and mechanisms of adsorption can be deduced from time-concentration data derived from sorbate-sorbent interaction prior to equilibrium [41, 42]. The initial adsorption rate and half-life were also calculated (eq. (6) and (7)) and the models were validated using the sum of square of errors (SSE) (eq. (8)) [40–42]. The kinetic parameters obtained from the models are presented in Table 2.

$$q_t = q_e (1 - e^{-K_{id}t})$$

(3)

$$q_t = \frac{q_e^2 K_{id}t}{q_e K_{id}t + 1}$$

(4)

$$q_t = K_{id} t^{1/2} + C$$

(5)

$$h = K_2 q_e^2$$

(6)

$$t_{50} = \frac{1}{K_2 q_e}$$

(7)

Table 2

<table>
<thead>
<tr>
<th>Adsorption kinetics</th>
<th>Parameter</th>
<th>ARVD Efavirenz (EFV)</th>
<th>Nevirapine (NVP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First order</td>
<td>Predicted $q_e$ (mg/g)</td>
<td>1.193</td>
<td>3.269</td>
</tr>
<tr>
<td></td>
<td>Experimental $q_e$ (mg/g)</td>
<td>1.956</td>
<td>4.717</td>
</tr>
<tr>
<td></td>
<td>$K_1$ (1/min)</td>
<td>1.017</td>
<td>1.000</td>
</tr>
<tr>
<td></td>
<td>SSE</td>
<td>1.180</td>
<td>1.237</td>
</tr>
<tr>
<td>Second order</td>
<td>Predicted $q_e$ (mg/g)</td>
<td>1.494</td>
<td>4.730</td>
</tr>
<tr>
<td></td>
<td>Experimental $q_e$ (mg/g)</td>
<td>1.956</td>
<td>4.717</td>
</tr>
<tr>
<td></td>
<td>$K_2$ (mg/g.min)</td>
<td>0.040</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>$h$ (mg/g.min)</td>
<td>0.090</td>
<td>0.220</td>
</tr>
<tr>
<td></td>
<td>Half-life ($t_{50}$)</td>
<td>16.670</td>
<td>21.140</td>
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<td></td>
<td>SSE</td>
<td>0.830</td>
<td>0.288</td>
</tr>
<tr>
<td>Weber-Morris</td>
<td>$K_d$ (mg/g.min^{1/2})</td>
<td>0.022</td>
<td>0.051</td>
</tr>
<tr>
<td>intraparticle diffusion</td>
<td>$C$</td>
<td>0.821</td>
<td>2.375</td>
</tr>
<tr>
<td></td>
<td>SSE</td>
<td>0.306</td>
<td>0.441</td>
</tr>
</tbody>
</table>

Fig. 2. Lagergren pseudo-first-order, pseudo-second-order and Weber-Morris intraparticle diffusion models of adsorption of (a) efavirenz and (b) nevirapine; onto graphene wool (Experimental conditions: Co = 5 mg/L; dosage = 10 mg per 5 mL, mixing rate = 200 rpm, $T = 25 \pm 1^\circ$ C). Error bars show ± relative standard deviation.
\[
\sum_{i=1}^{n} (q_{i,\text{exp}} - q_{i,\text{cal}})^2
\]  

(8)

Where \(q_i\) and \(q_e\) are the amount of adsorbate sorbed per mass of adsorbent (mg/g) at a time \((t)\) and equilibrium, respectively; \(K_i (1/\text{min})\) and \(K_2 (\text{g/mg} \times \text{min})\) are pseudo-first-order and pseudo-second-order rate constants, respectively; \(K_{di} (\text{mg/g} \times \text{min}^{1/2})\) and \(C (\text{mg/g})\) are the intraparticle diffusion rate constant and constant associated with boundary layer thickness, respectively; and \(h (\mu g/g \times \text{min})\) and \(t_{0.5}\) are the initial adsorption rate and half-life, respectively.

Table 3 revealed that the adsorption of efavirenz and nevirapine are best fitted to intraparticle diffusion and pseudo-second-order (PSO) kinetic models, given the lowest values of SSE. Fig. 2(a) revealed that EFV adsorption patterns exhibit multiple lineairties, suggesting that several steps governed the GW-EFV interaction. The boundary layer constant (C) is both greater than zero for GW-EFV and GW-NVP which suggests that other mechanisms aside from film diffusion occurred during adsorption of both sorbates [41]. The initial steps include bulk transport and film diffusion between the sorbent-solution boundary layer, followed by adsorption of EFV onto pores of GW [41, 43]. The SSE suggests that even though the intraparticle diffusion model also fit GW-NVP interaction, the pseudo-second-order (PSO) model best describes the adsorption pathway for nevirapine (Fig. 2b). This suggests that some degree of chemical adsorption took place and contributed significantly to the adsorption rate and mechanism for NVP uptake. The predicted and experimental amount adsorbed \(q_e\) for nevirapine are of similar magnitude (47.3 and 47.2), which further confirms that PSO best fits GW-NVP interaction.

The initial steepness observed in Fig. 2 in the first 240 min of contact, reflects fast initial adsorption due to availability of GW sorption sites to the respective ARVDs, before a period of slow adsorption as available sites diminish, and equilibrium is reached after site saturation. The GW-EFV and GW-NVP reached equilibrium after 600 min and 360 min, respectively. The initial rate constant \((h)\) reveals that the rate of adsorption of nevirapine is faster than efavirenz in the first 120 mins (Table 2), which could be attributed to lower molecular weight (size), availability of lone electron pairs, and π-electrons for binding interactions with GW. However, the PSO overall rate constant \((K_2)\) suggests that EFV had a faster diffusion-controlled rate of adsorption, which was faster than the rate of chemisorption governing the adsorption of NVP [42, 44].

### 3.3. Adsorption isotherm

Adsorption isotherms provide vital information on the nature of the interaction between sorbates and sorbents, especially the amount of analyte adsorbed, and the amount unadsorbed after equilibrium is reached [40]. Linear regression and nonlinear isotherm models such as Linear (eq. (9)), Freundlich (eq. (10)), Langmuir (eq. (11)), and Sips model (eq. (13)) were used to fit adsorption experimental data. The sum of square of errors (SSE) (eq. (8)) was used to test all models used in this study [44, 45].

Table 3

<table>
<thead>
<tr>
<th>Isotherm model</th>
<th>Coefficient</th>
<th>Efavirenz</th>
<th>Nevirapine</th>
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<tbody>
<tr>
<td>Linear</td>
<td>(K_d , (L/g))</td>
<td>1.482</td>
<td>2.544</td>
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<tr>
<td></td>
<td>SSE</td>
<td>0.093</td>
<td>0.091</td>
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<tr>
<td>Freundlich</td>
<td>(N)</td>
<td>1.263</td>
<td>0.902</td>
</tr>
<tr>
<td></td>
<td>(K_f)</td>
<td>1.269</td>
<td>2.569</td>
</tr>
<tr>
<td></td>
<td>SSE</td>
<td>1.295</td>
<td>0.038</td>
</tr>
<tr>
<td>Langmuir</td>
<td>(q_{\text{max}} , (mg/g))</td>
<td>8.99e2</td>
<td>25.547</td>
</tr>
<tr>
<td></td>
<td>(K_l , (L/mg))</td>
<td>0.002</td>
<td>0.119</td>
</tr>
<tr>
<td></td>
<td>(R_l)</td>
<td>0.940</td>
<td>0.248</td>
</tr>
<tr>
<td></td>
<td>SSE</td>
<td>1.370</td>
<td>0.042</td>
</tr>
<tr>
<td>Sips</td>
<td>(m)</td>
<td>23.503</td>
<td>0.945</td>
</tr>
<tr>
<td>(Freundlich-Langmuir)</td>
<td>(K_c , (L/mg))</td>
<td>1.065</td>
<td>0.058</td>
</tr>
<tr>
<td></td>
<td>(q_{\text{max}} , (mg/g))</td>
<td>4.408</td>
<td>48.313</td>
</tr>
<tr>
<td></td>
<td>SSE</td>
<td>1.263</td>
<td>0.039</td>
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Fig. 3. Plots of sorption isotherm model for (a) efavirenz (EFV) adsorption and (b) nevirapine adsorption onto GW adsorbent (Experimental conditions: \(C_0 = 1 - 20 \, \text{mg/L}; \text{dosage} = 10 \, \text{mg per 5 mL}; \text{mixing rate} = 200 \, \text{rpm}; \, T = 25 \pm 1 \, ^\circ\text{C}\). Error bars show ± relative standard deviation.
heterogeneity; \( q_{\text{max}} \) (mg/g) and \( K_L \) (L/mg) are the Langmuir maximum adsorption capacity and Langmuir constant associated with solute–surface interaction energy, respectively; \( K_F \) (L/mg) and \( q_{\text{max}} \) (mg/g) are Sips isotherm model constants and maximum adsorption capacity and \( m_s \) is Sips isotherm exponent; \( q_s \) is the solid-phase concentration (mg/g), \( C_s \) is the liquid phase equilibrium concentration (mg/L), and \( K_L \) (L/g) is the sorption distribution coefficient [22, 46]. The value of the separation factor \( R_L \) (eq. (12)) provides important information about the nature of adsorption; \( R_L < 1 \) = favourable adsorption; \( R_L > 1 \) = unfavourable adsorption; \( R_L = 1 \) = linear adsorption; \( R_L = 0 \) = irreversible [37, 38].

The one, two, and three-parameter models used in this study provided useful insight into the nature and mechanism of adsorption of the selected ARVDs by graphene wool; and nonlinear regression analysis of experimental data using equations (9 - 13), have been reported to provide a more accurate fit than linear regression [44, 45]. Isotherm data for EFV was best fitted by Sips isotherm model with least SSE < 1.27, while NVP was best described by a multilayer adsorption mechanism depicted by Freundlich model with SSE < 0.039, respectively (Fig. 3, Table 3).

Sips is a hybrid of the Langmuir and Freundlich model and describes heterogeneous adsorption systems [45]. Thus, given that it best fit EFV adsorption, this implies that the interaction of EFV with GW is complex and highly heterogeneous, which accounts for the comparatively high heterogeneity (\( N \) & \( m_s \)) index (Table 3). Basic models such as Freundlich and Langmuir would not completely describe the EFV sorption mechanism, due to limitations caused by increased adsorbate concentration and the nature of solute normally associated with the Freundlich model [45, 46]. Therefore, at low adsorbate concentration, the Sips model could reduce to Freundlich model (multilayer adsorption), and at high concentration of adsorbate, it predicts Langmuir model (monolayer adsorption).

The solute–surface interaction energy (\( K_L \)), Sips maximum adsorption capacity (\( q_{\text{ads}} \)) and adsorption capacities (\( K_F \) & \( K_L \)) revealed that NVP has stronger binding strength and higher sorption capacity onto GW adsorbents, while GW-EFV interaction is mainly dominated by weak van der Waals’ and hydrophobic bonding interactions considering its hydrophobicity (LogKow) and structure (Table 1).

### 3.3.1. Plausible sorbent-sorbate mechanism of interaction

The mechanism of interaction between adsorbents and adsorbates is often influenced by the moieties/functional groups and molecular/electronic conformation of the adsorbents and adsorbates. The bulky molecular nature of organic compounds including pharmaceuticals often leads to several competing interactions. In this study, the Sips model described the adsorption of EFV and NVP onto GW well, which affirms the existence of complex interactions, which can be influenced by concentrations of target compounds, solution’s pH, and temperature of the system. The electron pairs on the nitrogen atom in the pyridine aromatic structure present in nevirapine, suggest possible covalent bonding interactions between NVP and electrophiles without disruption of the aromatic ring of NVP. This provided a plausible explanation for the stronger interaction between GW-NVP than GW-EFV as presented by isotherm data (Table 3).

Other non-covalent bonding interactions are probable between GW-EFV and GW-NVP, and they include binding mechanisms such as \( \pi-\pi \) stacking, hydrogen bonding, van der Waals and hydrophobic bonding (Fig. 4), which have been reported to control the adsorption of several organic pollutants by graphene-based materials [47-49]. Furthermore, the presence of electronegative atoms such as fluoride, chlorine, and nitrogen in the target compounds, as well as the proton-rich structure of graphene; indicate that electrostatic attraction and repulsion in ionic aqueous medium cannot be ruled out, especially under varying pH [50].

### Table 4

<table>
<thead>
<tr>
<th>Sorbates</th>
<th>( K_{L\text{ads}} )</th>
<th>( N_{ads} )</th>
<th>( K_{F\text{ads}} )</th>
<th>SSE</th>
<th>( R^2 )</th>
<th>( *H )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efavirenz</td>
<td>0.70</td>
<td>1.26</td>
<td>4.39</td>
<td>1.50</td>
<td>0.992</td>
<td>0.29</td>
</tr>
<tr>
<td>Nevirapine</td>
<td>4.29</td>
<td>0.90</td>
<td>1.21</td>
<td>0.17</td>
<td>0.997</td>
<td>0.74</td>
</tr>
</tbody>
</table>

\( *H \): Sorption–desorption hysteresis index, \( H = N_{\text{ads}}/N_{\text{des}} \); \( N_{\text{ads}} \): Freundlich adsorption intensity, \( N_{\text{des}} \): Freundlich desorption intensity.
5. Thermodynamic parameters for adsorption of efavirenz and nevirapine onto graphene wool (GW).

Table 5

<table>
<thead>
<tr>
<th>Temperature (K)</th>
<th>ln b</th>
<th>$\Delta G$ (kJ/mol)</th>
<th>EFV $\Delta H$ (kJ/mol)</th>
<th>$\Delta S$ (kJ/mol.K)</th>
<th>ln b</th>
<th>$\Delta G$ (kJ/mol)</th>
<th>NVP $\Delta H$ (kJ/mol)</th>
<th>$\Delta S$ (kJ/mol.K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>298</td>
<td>6.45</td>
<td>-15.98</td>
<td></td>
<td></td>
<td>10.36</td>
<td>-25.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>308</td>
<td>11.52</td>
<td>-29.50</td>
<td>208.80</td>
<td>0.76</td>
<td>10.34</td>
<td>-26.48</td>
<td></td>
<td>-1.97</td>
</tr>
<tr>
<td>318</td>
<td>11.65</td>
<td>-30.80</td>
<td></td>
<td></td>
<td>10.31</td>
<td>-27.26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.4. Desorption isotherm and hysteresis

The release and subsequent recontamination potential of adsorbed compounds are often evaluated by desorption studies. Thus, evaluating the fraction of adsorbed EFV and NVP, that can desorb into an aqueous solution by reaching a new equilibrium is vital for GW industrial application and decontamination processes [41]. Hysteresis index ($H$), which is a measure of the irreversibility of the sorption process, was calculated for GW-EFV and GW-NVP interactions. Table 4 revealed $H$-index values for both ARVDs were greater than zero ($1/N_{ads} > 1/N_{ass}$), which implies that some degree of sorption-desorption hysteresis occurred [51].

The calculated hysteresis index was greater in GW-NVP, which suggests a higher tendency for irreversible sorption (hysteresis) in GW-NVP sorption-desorption interaction (Table 4). This can be attributed to stronger binding strength and possible sorption site defects and entrapment of adsorbed molecules [41, 52]. Experimental data revealed that more of NVP was adsorbed as clearly observed in the isotherm (Fig. 5). This can be attributed to stronger binding strength between nevirapine and GW (Fig. 5).

3.5. Effect of pH on adsorption of selected ARVDs.

The need to study the influence of pH on the adsorption of organic pollutants including PPCPs, in contaminated aqueous solution is germane towards predicting optimum process conditions. The presence of specific moieties such as -OH, -COOH, -NH groups in many pharmaceutical products ensures protein-binding and transport in blood for pharmacological action and therapeutic effects [53], however, these functionalities make them susceptible to deprotonation under variable pH conditions. Furthermore, pKₐ values of efavirenz and nevirapine are presented in Table 1, and many notable reports have affirmed that the acid dissociation constant (pKₐ) largely influences the effect of pH on sorption processes of PPCPs, as pH range determines when the bulky compound is cationic or anionic in aqueous solution [54-56]. Generally, solution pH alters the surface properties of adsorbents and speciation of the compounds in the solution.

Efavirenz becomes anionic at a pH range beyond its dual pKₐ values of 10.20 and 12.52, which is mainly responsible for the steep decline in

Table 6

Calculated interaction and binding energies of ARVDs onto various forms of graphene sheets.

<table>
<thead>
<tr>
<th>Adduct</th>
<th>$\Delta E_{int}$</th>
<th>$\Delta E_{tot}$</th>
<th>Inter-molecular delocalized electrons</th>
</tr>
</thead>
<tbody>
<tr>
<td>NVP</td>
<td></td>
<td></td>
<td>Total Dispersion H-bonds</td>
</tr>
<tr>
<td>GW−NVP</td>
<td>-117.15</td>
<td>-104.27</td>
<td>0.94</td>
</tr>
<tr>
<td>GW−NVP</td>
<td>-99.66</td>
<td>-93.01</td>
<td>0.91</td>
</tr>
<tr>
<td>GW−NVP (covalent)</td>
<td>-95.10</td>
<td>+113.97</td>
<td></td>
</tr>
<tr>
<td>GW(OH)−NVP</td>
<td>-149.49</td>
<td>-127.40</td>
<td>1.17</td>
</tr>
<tr>
<td>GW(OH)−NVP</td>
<td>-149.41</td>
<td>-126.23</td>
<td></td>
</tr>
<tr>
<td>GW(O)−NVP</td>
<td>-100.04</td>
<td>-95.81</td>
<td></td>
</tr>
<tr>
<td>EFV</td>
<td></td>
<td></td>
<td>Total Dispersion H-bonds</td>
</tr>
<tr>
<td>GW−EFV-H₂</td>
<td>-117.53</td>
<td>-112.30</td>
<td>1.01</td>
</tr>
<tr>
<td>GW−EFV²</td>
<td>-105.48</td>
<td>-94.94</td>
<td>0.97</td>
</tr>
<tr>
<td>GW(OH)−EFV-H₂</td>
<td>-145.39</td>
<td>-139.03</td>
<td></td>
</tr>
<tr>
<td>GW(O)−EFV²</td>
<td>-134.27</td>
<td>-125.69</td>
<td></td>
</tr>
<tr>
<td>GW(O)−EFV²</td>
<td>-95.23</td>
<td>-89.87</td>
<td>1.19</td>
</tr>
</tbody>
</table>

Fig. 5. Percentage adsorption and desorption of efavirenz (EFV) and nevirapine (NVP) by graphene wool (GW). Error bars show ± standard deviation, n = 5.

Fig. 6. Effect of pH on efavirenz and nevirapine adsorption onto graphene wool (Experimental conditions: $C_0 = 5$ mg/L; dosage = 10 mg per 10 mL solution, mixing rate = 200 rpm, $T = 25 ± 1$ °C, contact time: 24 h). Error bars show ± relative standard deviation.
controlled by other mechanism(s) (Waal charged sorbent). The pKa of NVP is 2.8 and it is mainly anionic at pHs repulsion between the anionic EFV and deprotonated GW (negatively adsorption in the basic medium. This is a result of electrostatic % adsorption in the basic medium. This is a result of electrostatic repulsion between the anionic NVP and negatively charged sorbent in acidic pH above its pKa (optimum at pH 5) because GW is protonated (positively charged) in an acidic medium, thus resulting in an attraction. A slight decline in adsorption at basic pH is due to weak electrostatic attraction and repulsion between the anionic protonated and deprotonated forms of EFV (EFV–H₂ and EFV²⁻, respectively) and NVP (NVP–H and NVP⁺, respectively) are shown in Table 1. The values of the adsorption equilibrium constant (ln b) and ΔG reveal that the impact of increasing temperature is more significant in GW-EFV interactions.

3.6. Adsorption thermodynamics and effect of temperature

Temperature plays a significant role in many physicochemical and biological processes. Adsorption thermodynamic parameters such as a change in enthalpy (ΔH), change in entropy (ΔS) and Gibbs free energy variation (ΔG), were derived from Van’t Hoff equation eq. (14) and (15). The adsorption equilibrium constant (b) was deduced from the isotherm data using Eq. (11), at varying temperatures and used for the Van’t Hoff plot (Fig. 7b) [50, 57]:

\[
\ln b = \frac{\Delta S}{R} - \frac{\Delta H}{RT}
\]

\[
\Delta G = -RT\ln b
\]

where ΔG is the change in the Gibbs free energy (kJ/mol); ΔH is the change in enthalpy (kJ/mol), and ΔS is the change in entropy (J/mol K). The value of b is derived from the Langmuir adsorption constant (K_L) by multiplying its value (in L/mg) by 1000 to convert the units to L/g, and then multiplied by the molar mass of the antiretroviral drug as stated in Table 1.

EFV adsorption is an endothermic process (ΔH) while NVP is exothermic (-ΔH) (Table 5). The negative ΔG and positive ΔS for GW-NVP and GW-EFV interactions confirm a spontaneous adsorption process, with an increase in spontaneity as temperature increases from 25 to 45 °C [21, 50]. The EFV adsorption diminished from 81% at room temperature to 74% at 45 °C, which could be attributed to a higher degree of disorderliness/randomness as temperature increased (Fig. 7). While NVP adsorption slightly improved from 84% at ambient temperature to 87% at 45 °C, which could be due to relatively lower net displacement in the sorbent-solution interphase (considering entropy values) [57].

3.7. Computational studies of GW-EFV and GW-NVP interactions

Computational modelling using Density Functional Theory (DFT) was performed to assist with the interpretation of experimental results, as well as to further explore the interactions between EFV, NVP, and graphene wool. Optimized structures of protonated and deprotonated forms of EFV (EFV–H₂ and EFV²⁻, respectively) and NVP (NVP–H and NVP⁺, respectively) are shown in Fig. 8. The sequence of (de)protonation for each structure was established through the selection of the lowest energy tautomers of all possible ionized states; interestingly, the acidic proton associated with pKa = 10.20 in EFV was found to be -C=–C-CH(CH₂)₂ – H₂1 in Fig. 8(a).

Since the synthesized graphene wool contains C–O functional groups (XPS spectrum, Fig. 1(b)), graphene wool was modelled using three different structures: i) a single 5 × 5 graphene sheet, C₇₅H₂₇, labelled as GW, ii) a graphene sheet functionalized with a single O atom, labelled as GW(O) and iii) a graphene sheet functionalized with a single OH group, labelled as GW(OH). Constructing adducts of the absorbates and the three different forms of GW allow for a careful investigation of the effects of dispersion, electrostatics, covalent and non-covalent interactions. In particular, the binding energy,

\[
\Delta E_{bind} = E(\text{adduct}) - E(\text{adsorbent}) - E(\text{adsorbate})
\]

calculates the adsorption energy relative to the lowest-energy, undeformed graphene sheet and adsorbates. In contrast, the interaction energy,

\[
\Delta E_i = E(\text{adduct}) - E'(\text{adsorbent}) - E'(\text{adsorbate})
\]
calculates the adsorption energy relative to the pre-organized, deformed molecules, where $E^{\circ}(\text{adsorbent})$ and $E^{\circ}(\text{adsorbate})$ are single-point structures in the geometry of the adsorbed adduct. The difference between $\Delta E_{\text{bind}}$ and $\Delta E_{\text{int}}$ is therefore the energy required to pre-organize the geometries of both adsorbent and adsorbate from their equilibrium structures to the conformations found in the adsorbed adduct, $\Delta E_{\text{bind}} = \Delta E_{\text{int}} + \Delta E_{\text{org}}(\text{adsorbent}) + \Delta E_{\text{org}}(\text{adsorbate})$.

The interactions between the ARVDs and the fully reduced GW are shown in Table 6. Interestingly, the neutral forms of NVP and EFV interact with a graphene sheet (Fig. 9) in a very comparable fashion ($\Delta E_{\text{int}} = -117.15$ and $-117.53$ kJ/mol, respectively) given the parameters of the model: no functional groups present on a monolayer graphene sheet and lack of any cooperative effects. In these conditions, similar interaction energy indicates that the degree of dispersion and electrostatic interaction between GW and adsorbate is very similar for both NVP and EFV. However, NVP needs to deform more than EFV in order to adsorb to the GW surface, resulting in a slightly stronger overall binding energy for the adsorption of EFV ($\Delta E_{\text{bind}} = -104.27$ and $-112.30$ kJ/mol for NVP and EFV, respectively). When both NVP and EFV are deprotonated, binding and interaction energies become less negative and indicate weaker adsorption. For instance, $\Delta E_{\text{int}} = -99.66$ kJ/mol in the GW--NVP$^-$ adduct, $+17.49$ kJ/mol higher than its neutral NVP-H counterpart. This observation therefore fully supports the reported experimental results, suggesting that negative charges on the deprotonated ARVDs leads to larger electrostatic repulsion with the generally electronegative graphene sheet.

It is plausible from the EFV--GW and NVP--GW interaction energies that these ARVDs adsorb to graphene primarily through dispersive interactions. The total number of electrons shared between each ARVD and the graphene sheet was modelled using FALDI and is tabulated in Table 6. NVP-H shares a surprisingly large total of 0.94 e$^-$ (almost a full electron) with GW. However, no single diatomic contact makes a significant contribution – of a total of 3128 inter-molecular diatomic contacts in the GW--NVP-H adduct, the largest diatomic contribution is a fractional 0.023 e$^-$ (3% of the total number of electrons shared), arising from a C--C contact (visualized in Fig. 10). Rather, the remarkable ability of graphene to delocalize electrons results in a strong dispersive interaction resulting from many, cumulative weak diatomic interactions. EFV-H$_2$ shares slightly more electrons with GW(O) (1.01 e$^-$) than NVP-H, indicating an even stronger dispersive interaction. On the other hand, the deprotonated forms of both ARVDs share slightly fewer electrons than their protonated counterparts. For instance, EFV$^{2-}$ shares slightly fewer electrons (0.97 e$^-$) with the monolayer graphene sheet than EFV-H$_2$.

Low and high pH conditions can be simulated by considering the
energies of NVP-H are stabilized when adsorbing to a GW**(OH)**, when the graphene sheet is functionalized in this manner, results in functionalized GW model, either as GW**(OH)** or GW**(O)**, respectively.

Fig. 10. Fig. 10. DFT lowest-energy adducts between reduced graphene oxide (GW, graphene sheet), protonated and deprotonated graphene oxide (GW**(OH)**, GW**(O)**) as absorbents and various (de)protonated forms of EFV and NVP.

When the graphene sheet is functionalized in this manner, results in Table 6 display a slightly different picture. The interaction and binding energies of NVP-H are stabilized when adsorbing to a GW**(OH)**, ΔEbind = −127.40 kJ/mol for GO**(OH)**⁻⁻NVP-H, −23.14 kJ/mol lower than for rGO⁻⁻NVP-H. Very similar values are observed for the deprotonated GW**(OH)**⁻⁻NVP⁻⁻ adduct. The origin of this stabilization in functionalized graphene relative to a reduced GW is clearly an OH⁻⁻N hydrogen-bond (Fig. 9(b)), which is confirmed with FALDI – an additional 0.17 e⁻ are shared amongst the relevant O, H, and N atoms (visualized in Fig. 10 (b)). At high pH conditions, the GW**(OH)** sheet is expected to be deprotonated to GW**(O)**. As a result, the GW**(O)**⁻⁻NVP⁻⁻ interaction is destabilized (ΔEbind = −95.81 kJ/mol) relative to GW**(OH)**⁻⁻NVP⁻⁻ and is very similar to the GW⁻⁻NVP⁻⁻ adduct, due to a lack of any significant H-bonding. Of particular note, however, is the interaction of EFV with graphene, was disregarded as unlikely due to extremely large binding energies at average covalent bond distances. The GW⁻⁻NVP⁻⁻ complex is characterized by moderately negative interaction energy (ΔEbind = −95.10 kJ/mol) but positive binding energy (ΔEbind = +113.97 kJ/mol), Table 6. Our modelling therefore suggests that the graphene⁻⁻NVP⁻⁻ covalent bond is attractive but requires large deformations in both adsorbate and absorbent in order to form. In addition, as we did not find any other structures which seems to support covalent bonding with graphene, it is highly probable that the observed covalent bond is a feature exclusive to the deprotonated form of NVP⁻⁻. It is very likely that a more complex model including e.g., multiple graphene layers, the inclusion of defects or increased graphene functionalization will further stabilize this covalent complex, but such modelling lies outside of the scope of this work.

Conclusion

A comprehensive risk-based assessment of graphene is currently unavailable; however, many researchers believe that the material does not pose a high health risk based on its composition, but it may pose a potential risk as a result of its thin and lightweight nature. Particularly, graphene in the particulate form could prove worrisome regarding inhalation risks. The physical structure of the graphene-based material and the fabrication method is therefore critical. With respect to graphene wool, the quartz wool substrate acts as solid support, assisting with immobilization of the graphene. This study revealed that graphene wool can be used as an effective adsorbent for the removal of antiretroviral drug contaminants, specifically efavirenz and nevirapine from aqueous solution. The Sips, Freundlich, pseudo-second-order, and intraparticle diffusion adsorption models best describe the sorption processes, and experimental variables such as pH and temperature only slightly influence nevirapine adsorption. This suggests that its interaction is majorly controlled by strong electronic interaction between moieties containing lone pairs leading to hydrogen bonding, and π⁻⁻π stacking between GW and NVP. It could be concluded that GW-EFV interaction is comparably weaker, with less hysteresis and higher desorption potential, and is controlled by hydrophobic and electrostatic interactions. Computational studies suggest that both GW⁻⁻EFV and GW⁻⁻NVP interactions are predominantly controlled by dispersive interactions, although specific (de)protonation of functional groups on the graphene layer can lead to significant additional stabilization through hydrogen bonds. This study presents the first experimental and computational investigation of the potential application of a graphene-based material (graphene wool) as an efficient next-generation sorbent for the removal of antiretroviral drug contamination in water. Furthermore, unlike most graphene generated in the form of flakes or powder, graphene wool provides a wool-like form that may be more suitable as a packing material for filters and other water polishing tools. The synthesis of graphene wool is facile and eco-friendly without extensive use of chemicals. Therefore, under appropriate operating conditions, the graphene wool adsorbent can potentially be utilized as a water polishing
The authors declare that there is no conflict of interest regarding the publication of this article.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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