Mobile Phone-based Colorimetric Detection of Perfluorocarboxylic Acids using Porphyрин Hosts

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Perfluorinated carboxylic acids (PFCAs) are widespread, potentially harmful, and difficult to detect pollutants. Here we investigate the use of three colorimetric porphyrin host molecules with chain lengths derived from different PFOA precursors as visual sensors for a range of different sized perfluorocarboxylates. We found that modifications to the length of the fluorinated chains led to subtleties in binding preferences and the resultant colorimetric (RGB) response. Host – guest interactions were investigated with UV-visible spectroscopy, and ImageJ software analysis was used to relate RGB information from digital photographs with binding and perceived colors. The CIE76 formula for color difference was used for the visual estimation of PFCA concentrations from generated color charts. Color responses were also parameterized to generate calibration curves from known concentrations of perfluorooctanoic acid so that total PFCA concentrations could be estimated with less than 20% error using a phone camera across a 10 ppb (parts per billion) – 16 ppm (parts per million) range.

Introduction

Perfluorocarboxylic acids (PFCAs) belong to a wide class of persistent fluorinated pollutants known as PFAS, or per- and polyfluoroalkyl substances. The ubiquitous presence of PFCAs arose from their use in the manufacturing of consumer goods, industrial supplies, and aqueous firefighting foams (AFFFs) prior to the year 2000.1 The strong fluorine – carbon bond (ca. 485 kJ mol⁻¹) provides PFCAs with desirable physical properties for non-stick, water repellent, and stain resistant materials, but also results in environmental persistence, transfer, and bioaccumulation.2 Typically, PFCA contamination includes molecules ranging from 4 – 14 carbons long, and physical characteristics such as solubility, hydrophobicity, and acidity vary greatly with each additional carbon (Table 1).3 The varying physical characteristics of PFAS means that people can be exposed to PFCAs through the respiratory, dermal, or digestive system.4 PFCA are not metabolised by the body, and the detrimental health impacts vary with chain length.4, 5 Perfluorooctanoic acid (PFOA) is well-known from its extensive use in the manufacturing of products like Teflon®, Gore-Tex® and AFFFs. PFOA is an eight-carbon chain perfluorinated carboxylic acid that is not produced in nature, yet is notably present in the blood serum of the majority of people living in industrialised countries (US median ca. 4 ng/mL).6 From a selection of almost 5000 different PFAS, long chain PFCAs are the most commonly observed in the environment.7 Since the regulation of PFOA, there has been an increasing occurrence of longer (C>9) PFCAs used in manufacturing, and consequently, an increased accumulation in the general population.8 Multiple studies have suggested that long chain PFCAs are potentially more biologically harmful than the banned PFOA due to their increased bioaccumulation.9, 10 To appropriately regulate exposure sources and mitigate potential health hazards, PFAS contamination must be promptly identified, which has driven the demand for rapid PFCA detection methods.

The currently available methods for PFCA analysis require operator training and careful handling to avoid cross contamination or sorption of the analyte during the extraction processes. For these reasons, an onsite, rapid colorimetric indicator that could give a total PFCA concentration would be able to streamline, direct, and potentially inform proceeding extensive PFAS analysis procedures. The concurrent detection of PFCAs across a broad size range is analytically challenging, because often samples are of low concentrations in complex matrices. The current method for PFCA analysis without derivatisation is liquid chromatography with tandem mass spectrometry (LC – MS/MS) using electrospray ionization.11 The use of solid phase extraction (SPE) has been coupled with chromatographic analysis to improve the limits of detection using preconcentration.12 C-18 sorbent has been shown useful for the detection of long chain PFCAs,13 but more recent developments have used specifically designed polymeric sorbents like Oasis®.14 These marketed “PFAS targeting” sorbents are typically mixed mode weak anion exchangers that can be paired with reverse phase LC.

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3 Electronic Supplementary Information (ESI) available: UV-Visible spectroscopic investigations, binding constants, colorimetric data and analysis, analysis of variance, calibration curves, and X-ray crystallography.
In previous work, we presented the fluorinated “picket fence” amido-porphyrin, 1, that bound PFOA to produce a color change detectable by eye from soil at concentrations as low as 3 ppm. Here, we present two new modifications to this host molecule (Figure 1) and investigate their responses to binding a range of PFCAs (Table 1). The host molecules have a preference to bind PFCAs over other common PFAS such as perfluorooctanoic sulfonic acid, allowing for development of a colorimetric method for PFCA screening. To further probe the applications of this chemistry for practical detection of PFCAs in field, we have investigated colorimetric analysis of the host molecules using a phone camera. The images contain red, green, and blue (RGB) information that can be extracted using software such as ImageJ or ColorX, allowing an untrained observer to utilise a smartphone for rapid semi-quantitative chemical analysis.

**Host Molecule Structures**

![Host Molecule Structures](image)

**PFCA Structure**

*Carboxylic acid head*  
Perfluorinated tail  
Perfluorocarboxylate

Chemical state is determined by temperature (MP/BP), and $pK_a$. Distribution in the environment will be influenced by solubility, vapor pressure, and distribution coefficients.

*Short chain PFCA* : $n = 4 \cdot 7$; $C_4F_7COOH \cdot C_7F_{15}COOH$  
*Long chain PFCA* : $n = 8 \cdot 12$; $C_8F_{17}COOH \cdot C_{11}F_{23}COOH$

**Fig. 1** Naming, classification, and physical properties of PFCA guest molecules.
Materials and methods

Materials
Perfluorobutanoic acid (PFBA; CAS# 375-22-4, 98%), perfluoropentanoic acid (PFPeA; CAS#2706-90-3, 97%), perfluorohexanoic acid (PFHxA; CAS#307-24-4, 97%), perfluoroheptanoic acid (PFHpA; CAS#375-85-9, 99%), perfluoroctanoic acid (PFOA; CAS# 335-67-1, 98%), perfluorononanoic acid (PFNA; CAS#375-95-1, 97%), perfluorodecanoic acid (PFDA; CAS#335-76-2, 98%), perfluoroundecanoic acid (PFUnDA; CAS#2058-94-8, 95%), and perfluorododecanoic acid (PFDoDA; CAS#307-55-1, 95%) were purchased from Sigma Aldrich Chemical Co. and used without further purification. Tetrakis(2-amino)porphyrin (0.010 g, 0.0007 M) was prepared according to the previously reported methods.15

Synthetic procedures
NMR experiments were performed on a Bruker Avance III NMR spectrometer operating at 600 MHz (1H), 150 MHz (13C) or 564 MHz (19F). The deuterated solvents (CDCl3 or CD2OD) were purchased from CIL Inc., and chemical shifts were recorded in ppm. Spectra were calibrated by reference to the residual solvent peak at δH 7.26 and δC 77.16 ppm for CDCl3 and δH 4.78 and δC 49.15 ppm for CD2OD. 19F NMR chemical shifts are relative to hexafluorobenzene in CDCl3 at δ = -163.9 ppm (external reference). Coupling constants (J) were recorded in Hz.

Host 1
α,α,α,α-5,10,15,20-Tetrakis[2-(perfluorooacylamino)phenyl]-21H,23H-porphyrin, host 1, was prepared according to the previously reported methods.15

Host 2
α,α,α,α-5,10,15,20-Tetrakis[2-(aminophenyl)]porphyrin (0.010 g, 1.5x10^-5 M) and pyridine (0.009 g, 1.2x10^-4 M) were combined in dry dichloromethane (10 mL) and stirred at ambient temperature for one hour. The solution of perfluorobutyl chloride (0.014 g, 6.0x10^-5 M) in dry dichloromethane (5 mL) was added dropwise. The mixture was stirred overnight at ambient temperature. Additional dichloromethane (5 mL) was added. The mixture was washed with water (10 mL), aqueous HCl (10 mL) and saturated aqueous NaHCO3 (10 mL). The organic phase was dried using Na2SO4 and the solvent was removed using rotory evaporation to yield a dark crystalline purple material. Crystals suitable for x-ray diffraction were grown by slow evaporation from dichloromethane. Crystals were dried under high vacuum. Single crystal X-ray crystallographic data have been deposited at the Cambridge Crystallographic Data Centre under deposition number CCDC 2128720 and can be accessed at https://www.ccdc.cam.ac.uk/structures/ (Yield = 0.15 g, ca. 66%). 1H NMR (CDCl3) δ 8.78(s, 8H), 8.62 (d, 4H, J = 8.2 Hz), 7.99 (d, 4H, J = 6.4 Hz), 7.93 (t, 4H, J = 7.6 Hz), 7.69 (s, 4H), 7.65 (t, 4H, J = 7.3 Hz), 2.69 (s, br, 2H). 19F NMR (CDCl3) δ 81.9 (3F), 122.3 (2F), -112.5 (2F). Host 3
Tricosafluorododecanoic acid (0.50 g, 8.1x10^-4 M) was combined with thionyl chloride (10.0 g, 8.0x10^-3 M) under an atmosphere of nitrogen and heated at reflux for 48 hours. The volatiles were removed under vacuum to yield a colorless solid to which dry toluene (20 mL) was added, and the volatiles were removed under vacuum. Dry dichloromethane (20 mL) was added, and the solution was stirred at ambient temperature. α,α,α,α,α,5,10,15,20-Tetrakis[2-(aminophenyl)]porphyrin (0.12 g, 1.8x10^-4 M) and pyridine (0.13 g, 1.5x10^-3 M) were combined in dry dichloromethane (20 mL) and stirred at ambient temperature for one hour. The solution of tricosafluorododecanoic chloride (0.45 g, 7.0x10^-4 M) in dry dichloromethane (20 mL) was added dropwise. The mixture was stirred overnight at ambient temperature. Additional dichloromethane (20 mL) was added. The mixture was washed with water (20 mL), aqueous HCl (2 x 20 mL), and saturated aqueous NaHCO3 (20 mL). The organic

Table 1 Naming and physical properties of PFCA guest molecules.

<table>
<thead>
<tr>
<th>Formula</th>
<th>Name</th>
<th>Acronym</th>
<th>MW</th>
<th>Log Koc</th>
<th>pKa</th>
<th>Vapor Pressure [Pa]</th>
<th>Water Solubility (20 °C, g/mL)</th>
<th>CMC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₃F₇COOH</td>
<td>Perfluorobutanoic acid</td>
<td>PFBA</td>
<td>231.03</td>
<td>1.9</td>
<td>0.4</td>
<td>1307</td>
<td>Miscible</td>
<td>162336</td>
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<tr>
<td>C₄F₉COOH</td>
<td>Perfluoropentanoic acid</td>
<td>PFPeA</td>
<td>263.04</td>
<td>1.4</td>
<td>0.4 - 0.6</td>
<td>1057</td>
<td>112.6</td>
<td>52695</td>
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<tr>
<td>C₅F₁₁COOH</td>
<td>Perfluoroheptanoic acid</td>
<td>PFHxA</td>
<td>313.05</td>
<td>1.3</td>
<td>0.7 - 0.9</td>
<td>457</td>
<td>21.7</td>
<td>24949-27994</td>
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<tr>
<td>C₆F₁₃COOH</td>
<td>Perfluoroheptanoic acid</td>
<td>PFHpA</td>
<td>363.05</td>
<td>1.6</td>
<td>1.7 - 2.1</td>
<td>158</td>
<td>4.2</td>
<td>10501</td>
</tr>
<tr>
<td>C₇F₁₇COOH</td>
<td>Perfluorooctanoic acid</td>
<td>PFOA</td>
<td>413.06</td>
<td>1.89</td>
<td>2.6 - 3.8</td>
<td>4</td>
<td>9.5</td>
<td>3777-4979</td>
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<tr>
<td>C₈F₁₉COOH</td>
<td>Perfluorononanoic acid</td>
<td>PFNA</td>
<td>463.07</td>
<td>2.36</td>
<td>3.69</td>
<td>-</td>
<td>1.3</td>
<td>9.5</td>
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<tr>
<td>C₉F₂₁COOH</td>
<td>Perfluorodecanoic acid</td>
<td>PFDA</td>
<td>513.08</td>
<td>2.76</td>
<td>2.96</td>
<td>2.6</td>
<td>0.2</td>
<td>9.5</td>
</tr>
<tr>
<td>C₁₀F₂₃COOH</td>
<td>Perfluoroundecanoic acid</td>
<td>PFUnDA</td>
<td>563.09</td>
<td>3.30</td>
<td>3.56</td>
<td>2.6</td>
<td>0.1</td>
<td>0.004</td>
</tr>
<tr>
<td>C₁₁F₂₅COOH</td>
<td>Perfluorododecanoic acid</td>
<td>PFDoDA</td>
<td>613.09</td>
<td>-</td>
<td>3.1</td>
<td>0.01</td>
<td>0.0007</td>
<td>-</td>
</tr>
</tbody>
</table>

Reference
18 *The Koc values reported in this table are based on a review of natural sediment studies.
phase was dried using Na₂SO₄ and the solvent was removed using rotary evaporation to yield a dark crystalline purple material (Yield 0.21 g, ca. 38%).¹¹H NMR (CDCl₃) δ 8.90 (s, 8H), 7.88 (m, 2H), 7.83 (m, 2H), 7.60 (t, 4H, J = 7.92 Hz), 7.18 (m, 4H), 7.13 (m, 4H), -2.67 (s, br, 2H). ¹³C NMR (CDCl₃) δ 155.2, 136.2, 136.1, 136.0, 135.5, 135.2, 135.0, 132.2, 132.1, 130.7, 130.6, 125.4, 121.7, 113.9, 109.8, 108.4. ¹⁹F NMR (CDCl₃) δ -80.7 (3F), -117.6 (2F), -121.7 – 122.6 (10F), -122.6 (6F), -126.0 (2F).

**Synthesis of tetrabutylammonium PFCA salts from acids**

This methodology was modified from a literature procedure.²⁶ The PFCA (0.0005 mol) was dissolved in warm deionised water with vigorous stirring. The solution was left to cool to ambient temperature before tetrabutylammonium hydroxide solution was added dropwise. If a precipitate formed, the solids were collected and washed with additional cold water. If there was no solid precipitate, the aqueous phase was extracted with dichloromethane, and the solvent was removed to yield a white solid. Solid products were twice recrystallized from dichloromethane by the addition of diethyl ether. TBAPF₃ was not reused throughout experiments. Materials that must be avoided to limit PFAS contributions to analysis include low density polyethylene and polytetrafluoroethylene (Teflon).

**General methods**

**General equipment and sampling considerations:** Ideal materials to be used when preparing PFAS samples include polypropylene, high density polyethylene, PVC, stainless steel, and silicone. Some analysis methods require the use of materials that may adsorb PFAS (primarily glass). Glassware use was limited when possible, and it was acknowledged that it could have a minor impact on the effective PFAS concentration during analysis. Glassware that once contained PFAS material was not reused throughout experiments. Materials that must be avoided to limit PFAS contributions to analysis include low density polyethylene and polytetrafluoroethylene.

**General method of UV-visible titration:** Path length of the quartz cell was 1 cm. For UV-visible (UV-vis) titrations, a stock solution of host was prepared (2.2 x 10⁻³ M) in dichloromethane and serially diluted to the required concentrations. Working solutions of tetrabutylammonium (TBA) anion salts or guest molecules were prepared in the same manner. Host – guest titrations used solutions of guest prepared in the working concentration of host solution, according to literature methodologies.²⁷

**Data analysis**

**General method for determination of association constants (K):** A UV-vis spectroscopic host – guest titration was performed to assess binding interactions. The experiment was designed according to the methodologies presented in Thordarson’s supramolecular titration guide.²⁷ A solution of host in dichloromethane was prepared. A solution of guest (PFCA) was then prepared in the host solution. Aliquots of guest in host solution are added to host solution and sequentially analysed until a known molar equivalent of guest has been added. Molar equivalents between 0.1 – 5 typically provide adequate information for modelling. The data was then used to simulate binding isotherms using [www.supramolecular.org](http://www.supramolecular.org).²⁸ The data was fitted to 1:1, 1:2 and 2:1 equilibria making no assumptions about the cooperativity of the binding interactions and modelled using different algorithms. These experiments mainly used the Nelder-Mead (Simplex) method because it is the most robust option. The L-BFGS-B (quasi-Newtonian) method, which has higher importance/constraints on K value estimates, was also tested, and provided similar results unless stated otherwise. A model was excluded if it could not be successfully fit, or there was a significantly large error (>15%) associated with the output.

**General method for RGB color analysis:**

A Puluz® 20 cm portable light tent with moderate and dispersed white LED lighting was used to photograph samples using an iPhone camera on a fixed tripod. The automatic flash settings were disabled so there was no reflective interference on the sample vials. Samples were photographed together to ensure lighting conditions and settings were consistent. The photographs were analysed using ImageJ software according to published methodologies. Triplet RGB values were chosen from areas of each sample at random to provide an “average” RGB value.²⁹ -³¹ These RGB values were used to produce an artificial color tile for visual comparison, or parameterized for modelling. To quantify a color difference, RGB values are transformed within the CIELab color space. The difference, expressed as ∆E, is determined by measuring the relative distance between two colors.³² The CIE76 algorithm transforms the L* a* b* coordinates according to the formula³³:

\[
\Delta E_{76} = \sqrt{\left( \Delta L^* \right)^2 + \left( \Delta a^* \right)^2 + \left( \Delta b^* \right)^2}
\]

The numeric ∆E value can be used to predict how the two colors are perceived by a standard observer; a ∆E > 2 is considered the minimum value to achieve a “just noticeable difference” (JND) (Table 2), which is the smallest difference required for an untrained observer to be able to determine two colors as being different.³⁴

<table>
<thead>
<tr>
<th>∆E</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1</td>
<td>Not perceived as different by the human eye</td>
</tr>
<tr>
<td>1 – 2</td>
<td>Perceptible upon close observation (JND)</td>
</tr>
<tr>
<td>2 – 10</td>
<td>Perceptible at a glance</td>
</tr>
<tr>
<td>11 – 49</td>
<td>Colors are more similar than opposite</td>
</tr>
<tr>
<td>49 – 100</td>
<td>Colors are more opposite than similar</td>
</tr>
</tbody>
</table>

**Results and discussion**

**Host Design and Derivatives**

The receptor molecules are designed to be modular combinations of a porphyrin chromophore, anion binding groups, and fluorophilic moieties. The preference to bind PFCAs appears to be due to the cumulative combination of both the carboxylate group and the fluorinated chain, as neutral fluorinated molecules and non-fluorinated carboxylates showed no colorimetric response.¹⁵ Other works have shown the fluorophilic attraction of isomers of the same receptor (1) are able to bind neutral fluorinated molecules, and demonstrate trends based on fluorophilic affinities.³⁵ In our previous investigation, it was found that the host molecule 1 had a strong affinity for PFOA. UV-Vis spectroscopic analysis
indicated host 1 bound PFOA with 1:1 binding in dichloromethane, with an association constant (K) in the range of ca. 7.50×10⁶ M⁻¹ (logK = 6.88). By design, the fluorinated chains of the host were an equivalent length to the guest molecule PFOA. To understand the influence the length of the fluorinated moiety had on binding PFCAs, three host molecules with varying degrees of fluorination derived from the PFAS precursors PFBA, PFOA, and PFDoDA were made and tested with the PFCAs.

**PFCA Guest Response Screening**

Experiments were performed at concentrations that were monitorable by eye, but binding interactions were investigated using UV-vis spectroscopy. UV-vis spectra were collected before and after the addition of 1 molar equivalent of each PFCA for each host, and the degree of complexation was noted by shifts in the Soret bands and formation of a peak at ca. 650 nm. The colors of each host – PFCA complex were photographed in a lightbox and analysed using ImageJ software RGB analysis. Pixels of the solution were chosen at random to provide RGB values. Multiple RGB values (n = 3) were recorded for each host – PFCA solution, and an average color produced. If there was a notable host – guest interaction for a particular PFCA (i.e., a strong affinity or bright colorimetric response), the host – guest binding was investigated with more rigorous UV-vis spectroscopy experiments, binding studies, and further color analyses.

**PFCA Guest Response – Host 2**

Host 2 provided a strong colorimetric response to all 9 PFCAs. This was evidenced in UV-vis spectroscopy where the shift of the λ_max was observed upon complexation. The Soret band of the host molecule 2 was observed at ca. 416 nm whilst the Soret band of the host – PFCA complexes were observed at ca. 444 nm (Figure S1). The addition of PFBA resulted in high conversion to the host – guest complex, while the addition of PFDoDA indicated the presence of significant quantities of both host and a host – guest complex. Although all the PFCA containing samples are visibly green, the green intensity decreases with increasing length of PFCA (Figure 2, Host 2). This was evidenced by the change in individual RGB channels; as an example, the differences between the R and G values were compared to highlight the change in the perceived color (Table S1).

**PFCA Guest Response – Host 1**

A colorimetric response was visible for all tested PFCAs when combined with host 1 in dichloromethane. The intensity and brightness of the green color was stronger for the short to medium chain length PFCAs. The formation of the host – guest Soret bands indicated binding across the entire range of PFCA guests (Figure S2), showing host 1, like host 2, to be a strong receptor for all the tested PFCAs. Like for host 2, the variability (green and red difference) between RGB values decreases as the length of the PFCA increases, detectable visually in solution primarily by the green hue or intensity (Table S2). The RGB color analysis showed the intensity and change in average color values were significant across the range of PFCAs (Figure 2, Host 1). When investigating potential binding preferences for PFCAs in the host molecules, it was found that host 1 could be the most consistently modelled. Triplicate host – guest addition titrations of host 1 and the TBA salts of the 9 different deprotonated PFCAs revealed the association constants are within a few orders of magnitude across the range of PFCA guests (Table S3), and the colorimetric response varies with chain length.

**PFCA Guest Response – Host 3**

Host 3 also provided a colorimetric response to all 9 PFCAs. The Soret band of host 3 is a different shape to the Soret bands of the host molecules 1 and 2; the shift in the Soret band of host 3 is less pronounced than that of 1 and 2, but the formation of a peak at ca. 650 nm upon binding is clearly distinguishable with all three host molecules. For this reason, absorbance values at the characteristic host – guest peak at ca. 650 nm are useful to show the response to each PFCA (Table S4), as the shifts in the Soret band for host 3 are less diagnostic of the color changes; λ_max 419 → ca. 426 nm (Figure S3). Unlike hosts 1 and 2, there was no obvious color trend for host 3 with the tested PFCAs. This may be because host 3 has a broadening of absorption bands upon complexation of PFCA complexes (Table S5).

Further spectroscopic investigations were undertaken to investigate binding from each host molecule with a single PFCA (PFOA), and each host molecule with its size matched PFCA guest (PFBA, PFOA, PFDoDA). The association constants for PFOA for host 2 (logK = 6.07 ± 0.7), 1 (logK = 6.25 ± 0.8), and 3 (logK = 6.22 ± 1.3) were of similar magnitudes, indicating a comparative affinity across the hosts. Hosts 2 and 1 demonstrated similar binding strengths with their size matched PFCAs, PFBA and PFOA (logK 6.12 ± 0.9, and logK 6.25 ± 0.9 respectively). The association constant for host 3 and PFDoDA (logK 5.68 ± 0.5) was an order of magnitude less. This may be due to the practicalities of an increased guest size adding physical hindrance. Titration data for host 3 and PFCA guests could also be modelled for 2:1 host – guest interactions when more than one equivalent of guest was present (Table S6). The nature of the 2:1 interaction was not characterized as the application of host 3 was intended to work with sub-stoichiometric amounts of guest for optimal colorimetric response.
After determining the variability of color changes from the host molecules for different PFCAs, we investigated the intensity of the color responses using PFOA across a range of concentrations for the purpose of creating a colorimetric calibration chart. An image in the RGB color space is composed of three data channels (R,G,B) ranging from 0 – 255. A black object has an RGB value of (0,0,0), while a white object has an RGB value of (255,255,255). The nature of RGB color information means that a lightening or darkening of a sample will result in a change that effects RGB values with equal direction and magnitude. This can mask changes that are due to color transformations when considering individual color channels independently. To better model the changes due to shifts in perceived color, the difference between individual RGB values can be compared. Colorimetric calibration charts were created using hosts 1, 2, and 3 (1.00x10⁻⁵ M) and solutions of PFOA (0–16 ppm, 4.00x10⁻⁶ M) and PFCAs (1 eq.) in dichloromethane, and the corresponding average color produced from ImageJ RGB analysis (left to right, host – control, PFBA – C₃F₇COOH → PFDoDA – C₁₁F₂₃COOH).

**Fig. 2** Photographs showing the colorimetric responses of each host (2.01x10⁻⁶ M) and PFCAs (1 eq.) in dichloromethane, and the corresponding average color produced from ImageJ RGB analysis (left to right, host – control, PFBA – C₃F₇COOH → PFDoDA – C₁₁F₂₃COOH).
M) in dichloromethane. Each solution of host (2 mL) was combined with an aliquot of each PFOA concentration (1 mL, final concentration: 0–1.33x10^{-5} M) and photographed under equivalent lighting conditions. The average color of each solution was then found using ImageJ RGB analysis, and a color chart was produced (Figure 3). At lower concentrations of PFOA, host 3 provided the largest change in RGB values, which can be seen visually. This is also observed in the ΔE values calculated using CIE76 algorithm for color difference. The ΔE values for each host and the lowest PFOA concentration (0.7 ppm) were 4, 11, and 24 for hosts 2, 1, and 3, respectively. Here, any sample containing PFOA at concentration > 0.7 ppm can be distinguished from the host visually or by RGB values (Figure 3, left). The differentiation and clustering of RGB values for samples containing PFOA demonstrate the suitability of this colorimetric response to be used as a threshold test. After host 3 showed both the most consistent response across the range of PFCAs, and the most discernible color change to PFOA, it was chosen as the sensor molecule of choice for further experiments.

Fig. 3 Comparison of RGB values for the host molecules (red) and any sample containing PFOA (green), and the corresponding color charts produced for the host molecules in dichloromethane across a range of PFOA concentrations (0–16 ppm). Plots of RGB values show that host 3 provides the most easily distinguishable color change for any sample containing PFOA, which is supported by the larger ΔE values produced from comparison of the host and each concentration of PFOA.
**Total PFCA detection**

Visual screening demonstrated that each host molecule 1, 2, and 3 provided a colorimetric response for PFCA of varying chain length. Although the association constants for host 1 suggest similar affinities for the range of tested PFCA, the colorimetric responses show a preferential “green” change for shorter chain PFCA. One-way factorial analysis of variance (ANOVA) was used to examine the dependence (or independence) of two or more factors affecting the dependent variable concurrently, in our case the change in absorption, the change in RGB response across the tested PFCA, or the change in RGB response across the different hosts. This analysis was not used to assess differences between the hosts, but the variability in the responses of each host across the different PFCA. Variability is the average squared deviations from the mean of a data set, which was applied to both the RGB information and the UV-vis absorption data. We can determine which host provides the most consistent response to the different sized PFCA using variability. The comparatively small variance in the RGB values collected for each PFCA and host 3 shows the colorimetric response to be more consistent than that of hosts 1 and 2 (host 1: $\sigma^2 = 110$, host 2: $\sigma^2 = 135$, host 3: $\sigma^2 = 15$) (Table S7). The analysis of absorbance observed at 650 nm shows that host 3 provides a more consistent response to the range of PFCA chain lengths (host 1: $\sigma^2 = 1.37 \times 10^{-4}$, host 2: $\sigma^2 = 8.98 \times 10^{-4}$, host 3: $\sigma^2 = 9.7 \times 10^{-5}$) (Table S8), which is also evidenced in the variance of the green value extracted from ImageJ analysis (host 1: $\sigma^2 = 32$, host 2: $\sigma^2 = 33$, host 3: $\sigma^2 = 14$) (Table S9). This means that from a visual perspective, host 3 has the lowest variability in colorimetric response across the different PFCA, and therefore is a practical choice for applications in determination of any PFCA in unknown samples; this was further probed using visual estimation and parameterization of RGB values to allow determination of total PFCA concentration.

**Visual matching of a PFCA sample**

To establish the feasibility of using a single host (host 3) as a visual sensor for different PFCA, a color chart based on the response of host 3 to known concentrations of PFOA was used to quantify other PFCA (Figure S5) – PFHxA is shown as an example. Samples of PFHxA (8 ppm) were prepared and combined with host 3 and the average color from triplicate experiments was compared to the calibration chart (Figure 4).

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**Fig. 4** Calibration chart generated for host 3 from the colorimetric response to PFOA using the 0 and 16 ppm RGB values, and color produced in response to PFHxA sample (8 ppm).

The RGB values for host 3 were collected at six concentrations of PFOA. To make a better comparison to the 8 ppm PFHxA sample, a color chart was generated by fitting 10 color points between the measured values for 0 and 16 ppm of PFOA. The generated colors were matched to the molar concentration of PFOA to give a scale of 12 concentrations (0 → 3.86x10^{-5} M). In this instance we were
matching a single PFCA, so the molar concentration was used for comparison; in situations where the sample is an unknown, or there is a mixture of PFCA\(_n\)s, the molecular weight cannot be accounted for and \textit{parts per notation equivalent to PFOA} must be used. When comparing the RGB values numerically using the CIE76 algorithm, the \(\Delta E\) values show which colors would be perceived as most similar. Each of the generated RGB values in the PFOA calibration chart were compared to the RGB values for the PFHxA sample, and the lowest value was used to determine the best visual match. The PFHxA sample (2.50x10\(^{-3}\) M, 8 ppm) was most similar visually to 2.72x10\(^{-3}\) M (ca. 9 ppm) PFOA. Using this method, the PFCA concentrations were estimated by eye with ca. 5.53x10\(^{-6}\) M standard error of the estimate (SEE), or ca. 9% absolute percentage error (APE).\(^{40,41}\) The visual estimation is a useful technique for matching a sample to a concentration range but will be limited in accuracy due to the color gradient maps, the sensitivity of the human eye, and the ability of each individual to perceive colors and changes in those colors. It was considered that a mobile phone camera and parameterization of the RGB values could be used to better predict PFCA concentrations.

**Parameterization of RGB values for determination of any PFCA**

Here we looked to parameterize RGB values to produce a calibration curve that could be used to predict a concentration of PFCA from an RGB parameter value. In the instance where a color change is occurring (e.g. red to green) instead of a change in colour intensity (e.g. red to darker red), the individual RGB channels may shift with different magnitude and direction.\(^{42}\) Thus, an increase in the average RGB value, (where \(\text{RGB} = \frac{(R+G+B)}{3}\)) does not always provide adequate information for determining a color change due to the presence of an analyte. For this reason, parameterization of the RGB values can be useful for interpreting results. The RGB parameter can be calculated using the RGB values of the “blank” host solution \(^{17}\) and the effective intensities of the individual RGB values of a sample \(^{43}\):

\[
\Delta R = |R_h - R_s|
\]

\[
\Delta G = |G_h - G_s|
\]

\[
\Delta B = |B_h - B_s|
\]  

(2)

Here, \(H\) indicates the values for the host solution, and \(S\) indicates the response for a sample containing a PFCA, so that \(\Delta R, \Delta G, \text{and} \Delta B\) give the color differences. The RGB parameter is the response due to the relative difference in the RGB intensities:

\[
\text{RGB Parameter} = \frac{\Delta R + \Delta G + \Delta B}{R_h + G_h + B_h}
\]  

(3)

The RGB values collected for each host with a range of known concentrations of PFOA in dichloromethane were parameterized and used to determine a relationship between concentration of analyte and RGB parameter values. The coefficients of determination, represented as the \(R^2\) value, were higher for hosts 1 and 3 \((R^2 = 0.9598\) and \(R^2 = 0.8945\) respectively, and host 2 \(R^2 = 0.6336\)), and host 3 had the lowest standard error \((\text{SE} = 3.10x10^{-6} \text{ M})\) (Figure S4). The relationship between RGB parameter values and concentrations of PFOA could now be tested for accuracy in determining the concentration of any PFCA.

**Colorimetric matching of mixed PFCA samples from RGB parameters**

The RGB parameters for the PFCA samples initially estimated using the visual method were predicted using equation (3). When the RGB parameter was used to estimate the PFCA concentrations from the PFOA calibration, they were predicted with ca. 2.12x10\(^{-7}\) M standard error, suggesting an improved accuracy from the \(\Delta E\) prediction method (Figure S5). After testing the colorimetric calibrations with the individual PFCA\(_n\)s the system was trialled with mixed PFCA \(_n\) samples across different concentration ranges. In this instance, we investigated the colorimetric responses for host 3 with “high” concentrations of PFCA (where \([H] < [G]\)), and “low” concentrations of PFCA (where \([H] > [G]\)). This is useful to trial for practical applications, as soil may be highly contaminated from the use of AFFS. In general, the RGB parameter value will increase with increasing concentrations of PFCA until the host has been saturated; beyond the saturation limit, RGB parameter values will be similar, and can only be used as a threshold indication for PFCA concentrations. Therefore RGB parameter calibration curves were established with host 3 (5.01x10\(^{-6}\) M) where the host : guest ratio did not exceed 1:1 \((0 – 6.0x10^{-6} \text{ M}; 0 – 40 \text{ ppb})\), and where the host concentration was exceeded by the guest concentration \((0 – 3.86x10^{-5} \text{ M}; 0 – 16 \text{ ppm})\) to mimic the two potential testing situations.

For the \([H] < [G]\) experiment, the PFOA generated calibration curve \((y=0.0401\ln(x)+0.2366, R^2 = 0.9677, \text{SE} = 1.64 \text{ ppm})\) (Figure S6) begins to level out at PFOA concentrations of 4.80x10\(^{-6}\) M (ca. 2 ppm, [H]:[G] = 1:0.75). Above this PFCA concentration, the RGB parameter values are the same because all the host has been bound (Figure S5). Thus, when testing a total PFCA concentration of 12 and 15 ppm the \(\Delta E\) value, \((\Delta E = 2)\), indicates that the colors would be perceived as the same and could not be determined as different from a 2 ppm sample (Table S10). When the total PFCA concentration was 0.2 ppm, that is, below the host saturation, the color could be used to estimate the concentration of total PFCA within 10% APE; \([\text{PFHxA}]_{\text{added}} = 0.20 \text{ ppm}, [\text{PFCA}]_{\text{predicted}} = 0.18 \text{ ppm equivalent to PFOA}\).
In the [H] > [G] experiment, the RGB parameter values had the greatest rate of change from 0 – 80 ppb PFOA and appeared to flatten between 40 – 80 ppb PFOA (Figure 5, inset). For this reason, in the second experiment, the regression was fit for a host : guest ratio of 0 – 0.8 (0 – 40 ppb). To probe the response to a mixture of PFCAs, samples containing combinations of PFHxA, PFHpA, and PFDA to give total PFCA concentrations of 7 – 28 ppb were prepared and the RGB responses were collected. The total PFCA concentration for a sample was calculated using the PFOA generated regression ($y = 0.0591\ln(x) + 0.037$, $R^2 = 0.9771$, SE = 2.72 ppb) (Figure 6). The concentrations of the mixed PFCA samples were predicted with ca. 0.6 ppb (1.48x10^-9 M) SEE.

![Fig. 5](image1.png)  
**Fig. 5** The RGB parameter values for different host : guest ratios; inset shows that below a host : guest ratio of 1:1 there is significant difference between individual RGB parameter values.

![Fig. 6](image2.png)  
**Fig. 6** Calibration curve produced from average RGB values collected for host 3 and 0 – 6x10^-8 M (0 – 40 ppb) PFOA. The PFOA samples are shown in green, and the PFCA samples RGB parameters are shown in orange.
The RGB parameters can differentiate between 0 and 1 ppb (1.88x10^{-9} M) PFOA numerically on the regression, but to assess the visual detectability, the ∆E values were calculated. When comparing the host 3 solution with each concentration of PFOA, the smallest value of ∆E was still greater than 2, which suggests all the PFOA concentrations could be differentiated visually from the host 3 solution by eye (Figure 7). Even samples of 1 – 5 ppb PFOA gave a color change that could be determined as different from the starting host solution visually (ΔE = 4–8) (Table S11). Based on this, a conservative visual limit of detection of 10 ppb PFCA should be considered for an untrained observer undertaking threshold analysis.

![Fig. 7 The ∆E values for PFOA and mixed PFCA samples when compared to the starting host 3 solution.](image)

Like the PFOA samples, the mixed PFCA samples could be determined as different from the host solution visually (average ∆E > 10). The concentrations estimated using the RGB parameters for the mixed PFCA samples also suggested the limit of detection to be above 10 ppb. The lowest concentration PFCA sample (7 ppb) was predicted to be ca. 11.1 ppb equivalent to PFOA (22% APE), whilst the higher concentration mixed PFCA samples (14.1 and 28.8 ppb) were predicted to be ca. 16.2 and 22.9 ppb equivalent to PFOA (2 and 16% APE respectively). This shows host 3 can be coupled with a phone camera to estimate a total concentration of mixed PFCA samples from RGB data with < 20% APE (1.79 ppb SE), or used visually for threshold detection of total PFCA concentrations above 10 ppb equivalent to PFOA.

**Conclusions**

We have investigated potential colorimetric total PFCA detection with three porphyrin host molecules. It was found that modifying the fluorinated alkyl chain lengths of the hosts led to subtleties in the colorimetric response. Hosts 1 and 2 demonstrated preferential colorimetric responses when binding of shorter chain PFCA, while host 3 had a more consistent response to a range of different sized PFCA. This made host 3 a more suitable sensor for total PFCA determination. The parameterization of RGB values extracted from a phone camera image allowed for colorimetric calibration to determine any PFCA samples. Here we have demonstrated the colorimetric detection of known and mixed PFCA samples between 10 ppb and 16 ppm. There is large scope for development in designing a practical colorimetric total PFCA sensor, and we have shown that modifications to sensors, concentrations, data processing, and experimental design can be optimized for targeted or “fit for purpose” detection.

**Author Contributions**


**Conflicts of interest**

The authors (C.M.T, M.C.B, N.L.K) have submitted a provisional patent related to this work (IP Australia application number 2021900529).

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