In Situ *Monitoring* of Lipid Removal from Model Fabric Surfaces

Supporting Information

James A. Barclay[A], Lydia G. Smith[A], Dalila Di Leva[A], Silvia Ruscigno[B], Clare S. Mahon[A], and Andrew Beeby[A*]
[A] Department of Chemistry, Durham University, DH1 3LE, United Kingdom
[B] Procter & Gamble, Newcastle Innovation Centre, Newcastle-Upon-Tyne, NE12 9TS, United Kingdom

Contact: Andrew.Beeby@Durham.ac.uk

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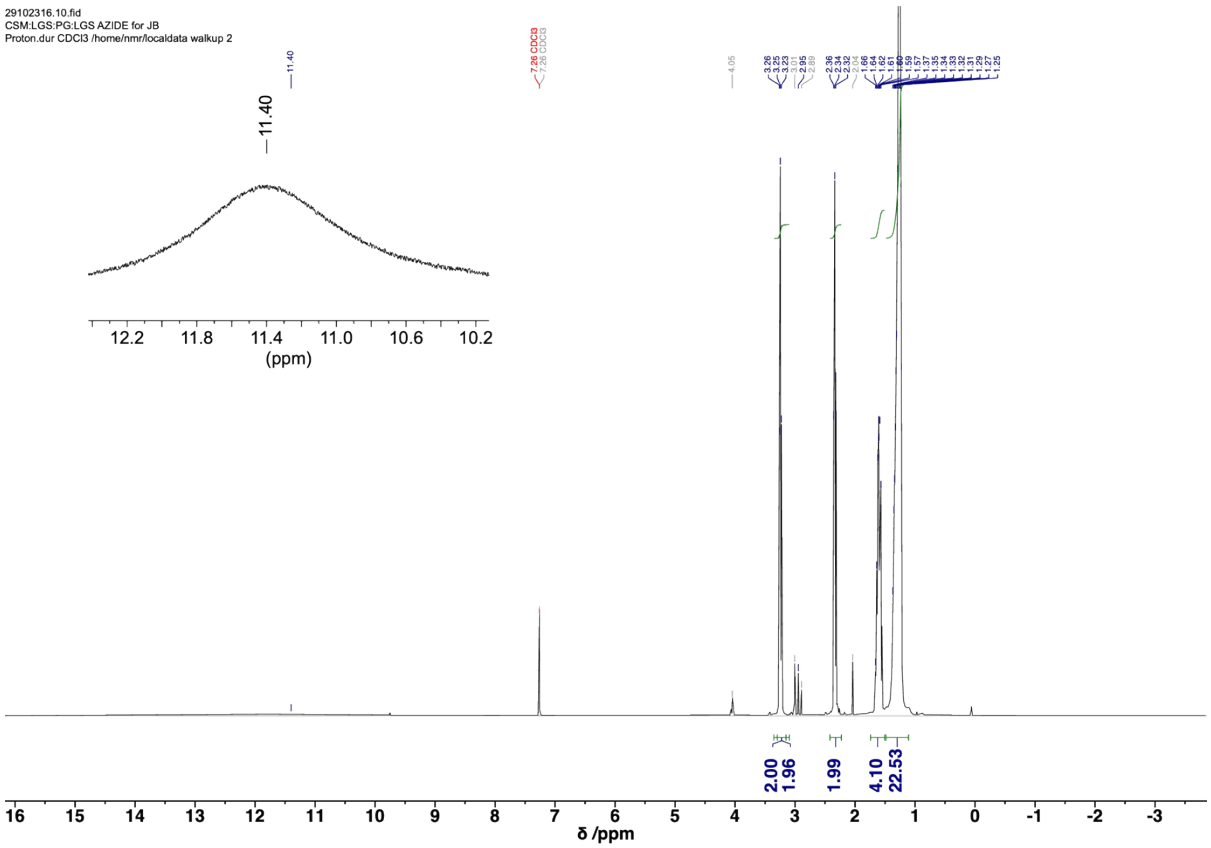
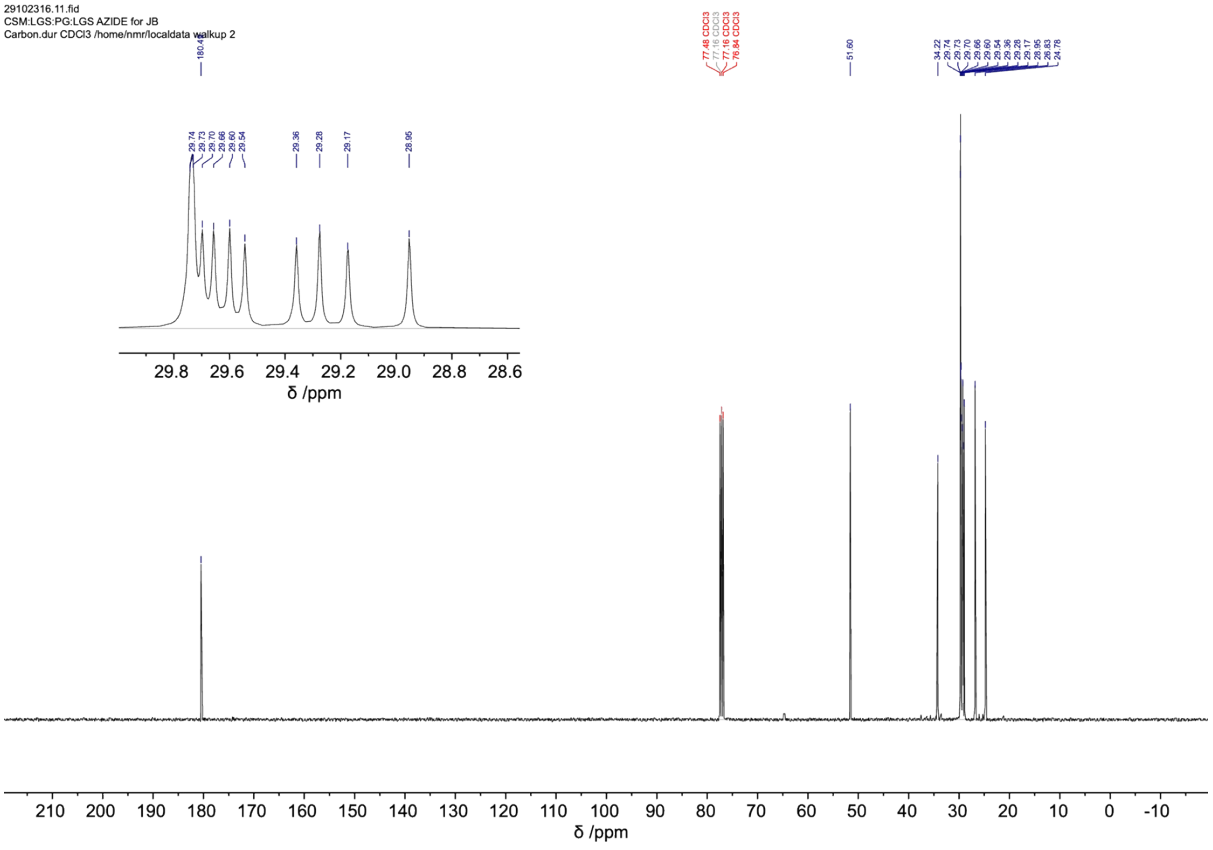
PREPARATION OF AZIDE LABELLED PALMITIC ACID

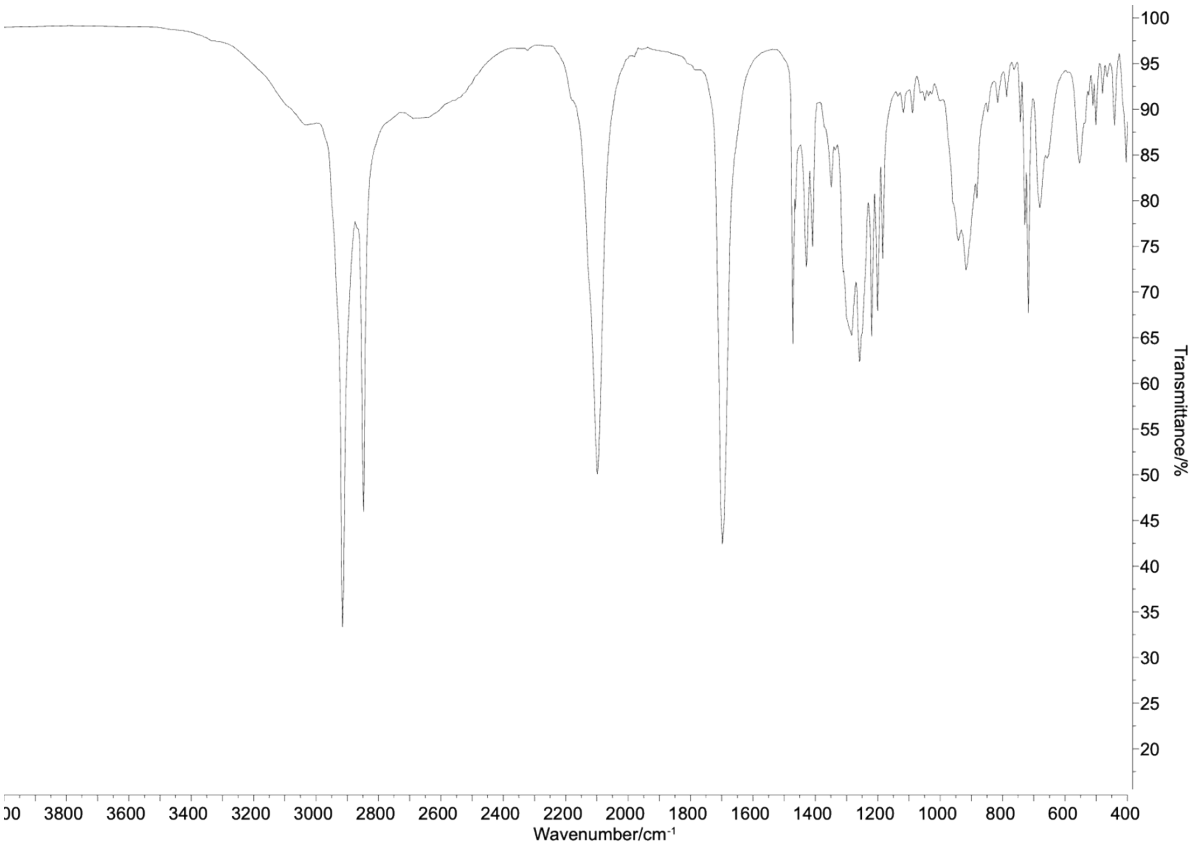
Synthesis adapted from literature.[1] 16-bromohexadecanoic acid (0.250 g, 0.746 mmol, 1.0 eq.) and NaN_3 (97 mg, 1.5 mmol, 2.0 eq.) were dissolved in DMF (30 mL) and the reaction mixture was stirred at 80°C for 16 h. The reaction mixture was cooled to room temperature and 1:1 EtOAc:H_2O (30 mL) was added. The mixture was extracted with EtOAc (3 × 30 mL) and the organic extracts were washed with $NaCl_{(aq)}$ (3 × 30 mL), dried over $MgSO_4$ and the solvent was removed *in vacuo* to yield a white solid (0.223 g). The crude product was purified by flash column chromatography (SiO_2,100% hexane → 100% EtOAc) to afford the product as white amorphous solid (0.189 g, 86%).

^{1}H NMR: (400 MHz, $CDCl_3$) δ 11.41 (s, 1H), 3.23 (t, *J* = 7.1 Hz, 2H), 2.32 (t, *J* = 7.6 Hz, 2H), 1.66 – 1.52 (m, 4H), 1.44 – 1.09 (m, 22H).**^{13}C{^{1}H} NMR:** (100 MHz, $CDCl_3$) δ 180.4, 51.4, 34.1, 29.74, 29.73, 29.70, 29.66, 29.60, 29.54, 29.36, 29.28, 29.17, 28.95, 26.68, 24.64**. LC-MS: [$C_{16}H_{30}N_3O_2$]$^-$** 296.48 found, **HR-MS:** $C_{16}H_{30}N_3O_2$ (calculated 296.2338) found 296.2326 (mass error -4.0509 ppm)**, FTIR-ATR:** 1472 (CH), 1698 (C=O), 2099 (N_3), 2828 (CH), 2915 (CH),

Spectral data is in agreement with previous reports.




29102316.11.fid
CSM:LGS:PG:LGS AZIDE for JB
Carbon.dur CDCl3 /home/nmr/localdata walkup 2
160.46
77.48 CDCl3
77.16 CDCl3
76.84 CDCl3
51.60
34.22
29.74
29.73
29.70
29.66
29.60
29.54
29.36
29.28
29.17
28.95
26.83
24.76
δ /ppm
δ /ppm


Transmittance/%
Wavenumber/cm-1

Flow Cell Hydrodynamic Characteristics

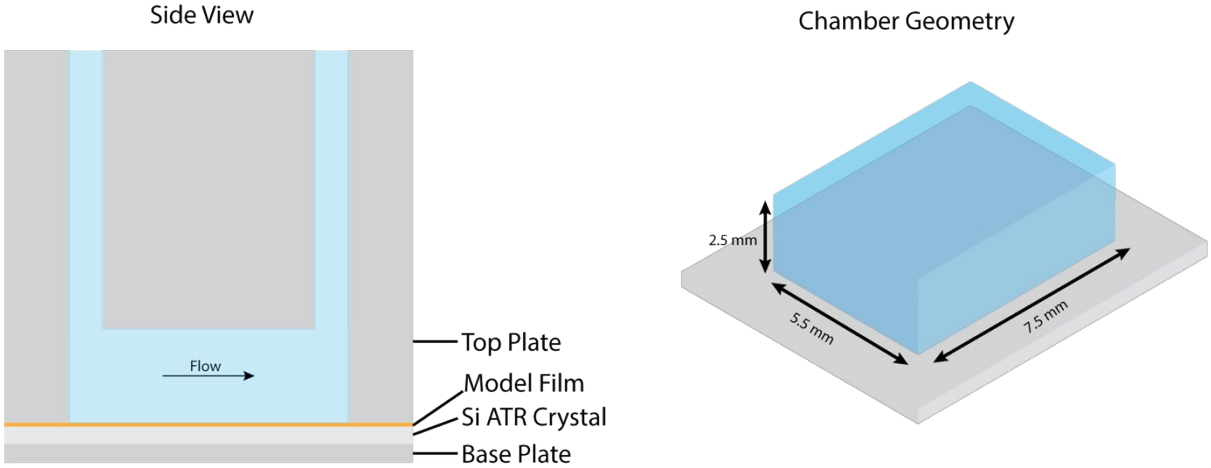


Figure S1: Side view of flow cell and flow chamber (left) and 3D geometry of the flow chamber.

The Reynolds number of the flow was calculated by using Equation S1:

$$Re = \frac{\rho v D_H}{\mu} \#(S1)$$

Where ρ is the density of the fluid (0.997 g mL^{-1} for water at 25 °C), D_H is the characteristic length of the system, and μ is the dynamic viscosity of the fluid (0.8891 mPa•s for water at 25 °C). The hydraulic diameter of a rectangular channel may be calculated from the channel dimensions using Equation S2:

$$D_H = \frac{2ab}{a+b} \#(S2)$$

Where a and b are the width and height of the channel respectively. For our flow channel, D_H = 3.4375 mm. The flow velocity, v, is obtained from the flow rate of our system, Q = 0.5 mL min^{-1}, or 8.33 mm^3 s^{-1}.

$$v = \frac{Q}{ab} \#(S3)$$

This gives a flow velocity of 0.606 mm s^{-1} for our system. Applying equation S1 to our system gives Re = 2.34 at 25 °C. We tested our cell up to 10 mL min^{-1}, the flow was found to be highly laminar at all tested flow rates.

```
%%%%Flow Cell Analyser %%%%
%%%James.A.Barclay%%%%
%Script to analyse FTIR Data generated by surfactant washoff cell

clc
clear
close all

%%%Data
Input%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%
%%% Put the path of the working directory below, this points the script to
%%% look for files in the correct place on your computer
cd('Directory');
% Please put your data file in a folder within the "InputData" folder in
% this directory. Put ONE .csv file in each folder, make a seperate folder
% for each run
Path = 'Figure 6/Figure 6A/';
%This section finds the .csv file within the input data folder and prepares
%variables for FTIR data, wavenumber and Time
%You will likely have to modify this to accomodate the input format of your
%spectrometer
Source = dir(fullfile(Path,'*.csv'));
File = fullfile(Path,Source.name);
Source.data = readmatrix(File);
Source.name = extractBefore(Source.name,".c");
Source.FTIR = Source.data(3:end,2:end);
Source.Wavenumber = Source.data(3:end,1);
Source.Time = Source.data(1,2:end);
Source.Time = Source.Time./60; %Converts time to minutes from seconds
OutputName = extractAfter(Path,'/');
OutputDir = mkdir(append('Output/',OutputName));
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%

%%%Data Setup and
Preprocessing%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%Chops off upper and lower portions of spectra for better fitting in region
%of interest
UpperThreshold = 2000;
LowerThreshold = 999;
WavenumberTruncated = Source.Wavenumber(LowerThreshold:UpperThreshold);
FTIRSmoothed = zeros(numel(Source.FTIR(1,:)),numel(Source.Wavenumber));
for k = 1:numel(Source.FTIR(1,:))
FTIRSmoothed(k,:) = smoothdata(Source.FTIR(:,k),"sgolay",5);
end
FTIRTruncated = FTIRSmoothed(:,LowerThreshold:UpperThreshold);
%loop performes baseline correction on all of the data in series using
% Backcor function (see backcor.m, this must be in the home directory or
% added to MATLAB Path).
Background = zeros(k,numel(WavenumberTruncated));
for k =1:numel(FTIRSmoothed(:,1))
   [EST] = backcor(WavenumberTruncated,FTIRTruncated(k,:), ...
     9, ...     %Polynomial Order
   0.01, ...  %Threshold
   'ah' ... %}   %Fitting Function choose from sh, ah,atq,stq
      );
   Background(k,:) = EST;
end
BackgroundSubtract = transpose(FTIRTruncated-Background);
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%

%%%Peak Analysis
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%Set regions of CH and CD bands to be integrated
CHRegion = 2700:2999;
```

```
CDRegion = 2050:2250;
CHRegion = 4000-LowerThreshold-CHRegion;
CDRegion = 4000-LowerThreshold-CDRegion;
%Extracts segement of background subtracted spectrum and integrates
CHExtracted = BackgroundSubtract(CHRegion,:);
CDExtracted = BackgroundSubtract(CDRegion,:);
CHIntegral(:,1) = trapz(CHExtracted);
CDIntegral(:,1) = trapz(CDExtracted);
%Normalise integral data to maximum input
CHRelIntegral =CHIntegral./max(CHIntegral);
CDRelIntegral =CDIntegral./max(CDIntegral);
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%

%%%Data
Plotting%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%
%Plots Raw data and saves output
n = numel(FTIRTruncated(:,1));
C = cbrewer2('Blues',2*n);
colororder(C(n:(2*n),:));
Source.FTIRZero = Source.FTIR-min(Source.FTIR);
plot(Source.Wavenumber,Source.FTIR,'LineWidth',1)
ax = gca;
set(gca, 'XDir','reverse')
ax.FontWeight = "bold";
ax.FontSize = 14;
xlim([1000 4000]);
xlabel('Wavenumber/cm^{-1}');
ylabel('Absorbance/AU');
ax.XAxis.LineWidth = 2.5;
ax.YAxis.LineWidth = 2.5;
saveas(gcf,append('Output/',OutputName,'/RawIR'),'png');

%Plots Background Corrected data and saves output
figure
colororder(C(n:(2*n),:));
plot(WavenumberTruncated,BackgroundSubtract,'LineWidth',1)
ax = gca;
set(gca, 'XDir','reverse')
ax.FontWeight = "bold";
ax.FontSize = 14;
xlabel('Wavenumber/cm^{-1}');
ylabel('Normalised Intensity/AU');
xlim([2000 3000])
ax.XAxis.LineWidth = 2.5;
ax.YAxis.LineWidth = 2.5;
saveas(gcf,append('Output/',OutputName,'/BackgroundCorrectedIR'),'svg');
figure

%Plots relative integrals over time
scatter(Source.Time,(CHRelIntegral),120,'filled','square')
hold on
scatter(Source.Time,(CDRelIntegral),120,'filled','square')

legend('CH Band','CD Band', 'Location','best')
ax = gca;
xlim([0 20])
ylim([0 1]);
ax.FontWeight = 'bold';
ax.FontSize = 14;
xlabel('Time/Min');
ylabel('Relative Intensity');
box on
ax.XAxis.LineWidth = 2.5;
ax.YAxis.LineWidth = 2.5;
saveas(gcf,append('Output/',OutputName,'/ExtractedWavelength'),'svg');
```

```
hold off

%Collates and writes .csv file containing integral outputs of spectra
OutputIntegrals = table(transpose(Source.Time),CHIntegral);
writetable(OutputIntegrals,append('Output/',OutputName,'/OutputData.csv'));
```

CONDITIONS USED FOR FLOW CELL EXPERIMENTS

Table S1 shows the conditions for washing solutions used in this work, along with their location in the main body of the text/ESI. All experiments were performed as to the general procedure detailed in the materials and methods. Representative spectra for lipid wash-off are provided for each washing solution.

Table S1: Breakdown of washing conditions used in this work.

Washing solution	[LAS] /ppm	[NI] /ppm	Additive	Figure Location
A	300	300	N/A	1, 6 A) i)
B	450	150	N/A	6 A) i)
C	150	450	N/A	6 A) i)
D	300	300	10 mM Na_2CO_3/$NaHCO_3$ Buffer, pH 9	6 A) ii)
E	300	300	10 mM Na_2CO_3/$NaHCO_3$ Buffer, pH 9, Lipex 200 L, 1 ppm	6 A) iii)
F	300	300	180 ppm $CaCl_2$	6 B) i)
G	600	0	180 ppm $CaCl_2$	6 B) ii)
H	0	600	180 ppm $CaCl_2$	6 B) iii)
I	300	300	180 ppm NaCl	S12
J	300	300	180 ppm $MgCl_2$	S12

REPRESENTATIVE WASH-OFF SPECTRA

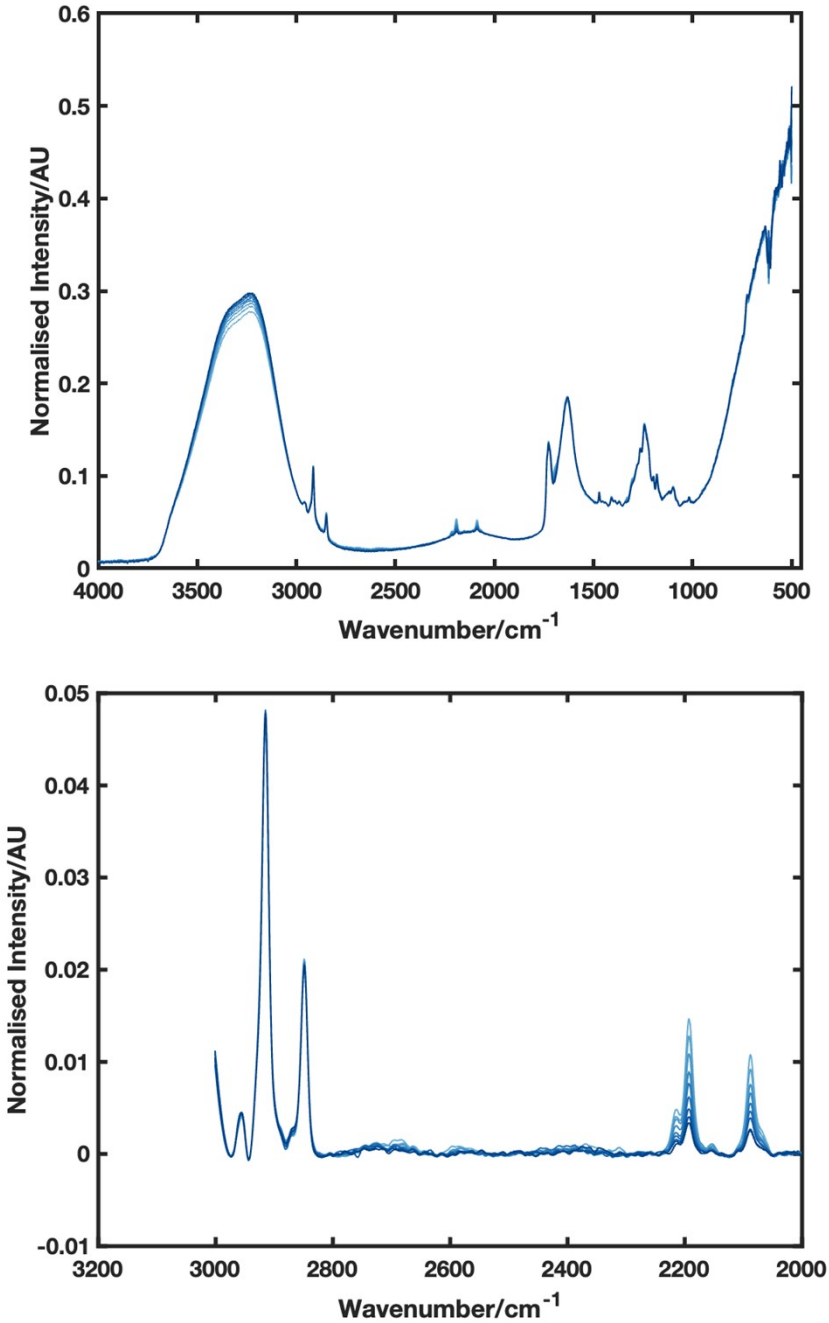


Figure S2: Representative Raw (top) and processed (bottom) spectra for wash-off of tripalmitin and palmitic acid-d using Solution A

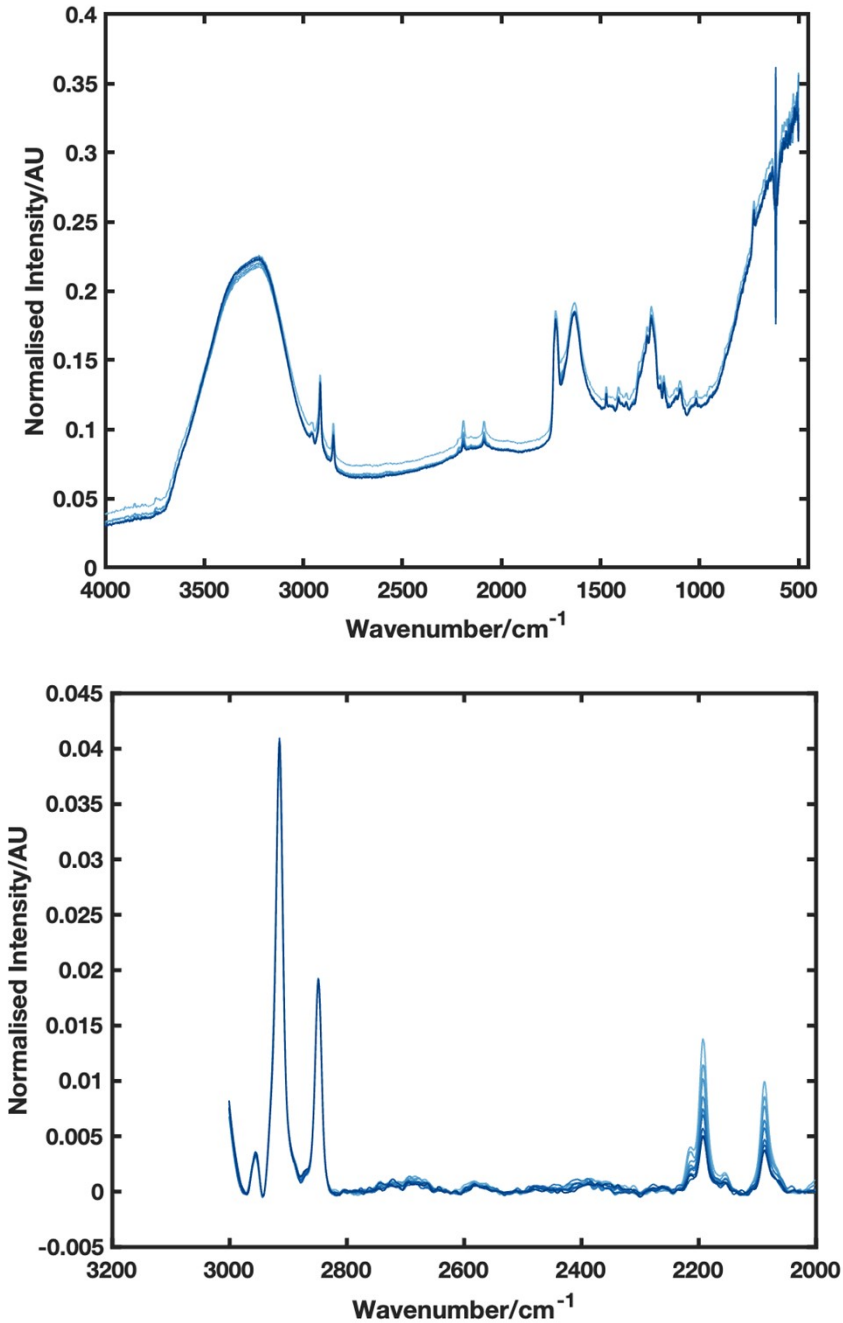


Figure S3: Representative Raw (top) and processed (bottom) spectra for wash-off of tripalmitin and palmitic acid-d using Solution B

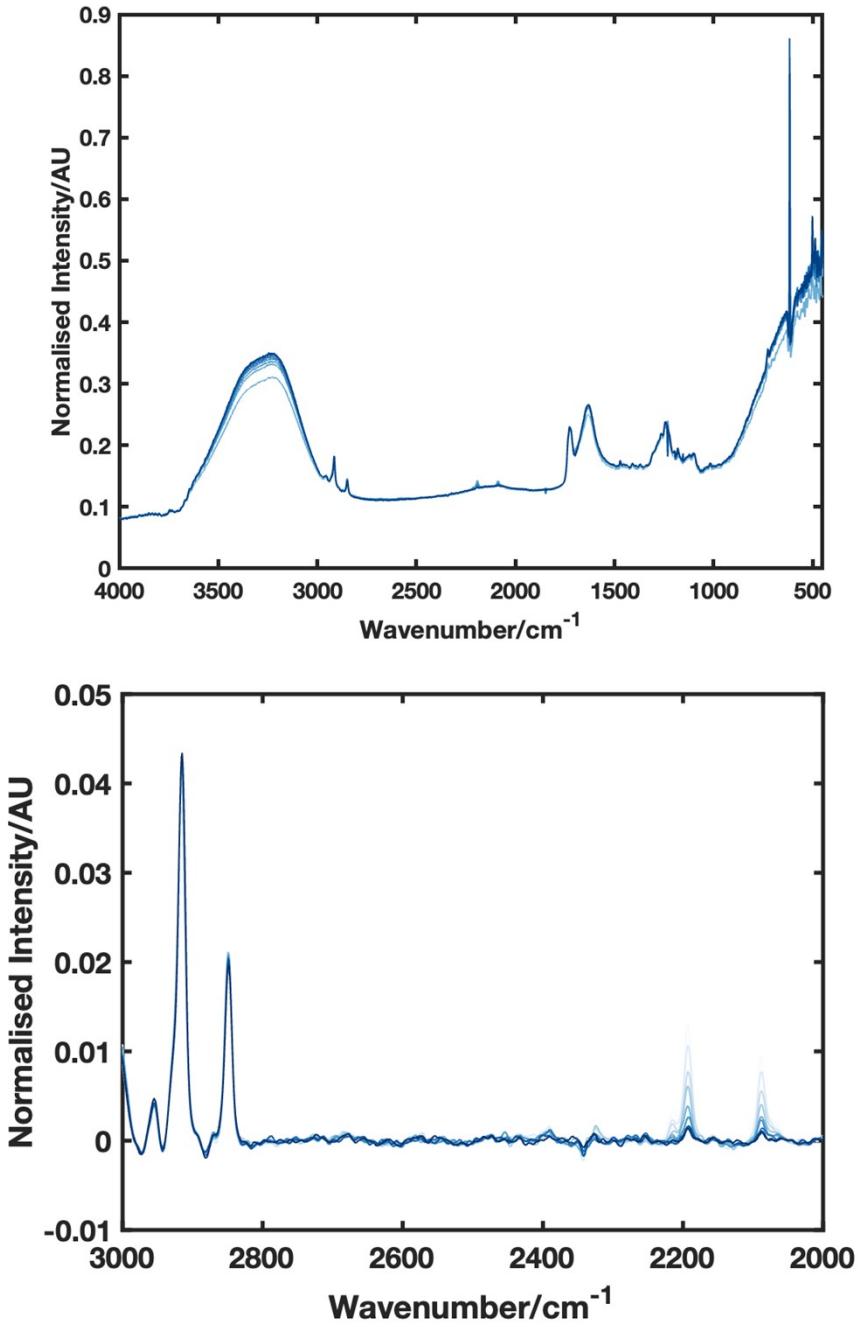


Figure S4: Representative Raw (top) and processed (bottom) spectra for wash-off of tripalmitin and palmitic acid-d using Solution C

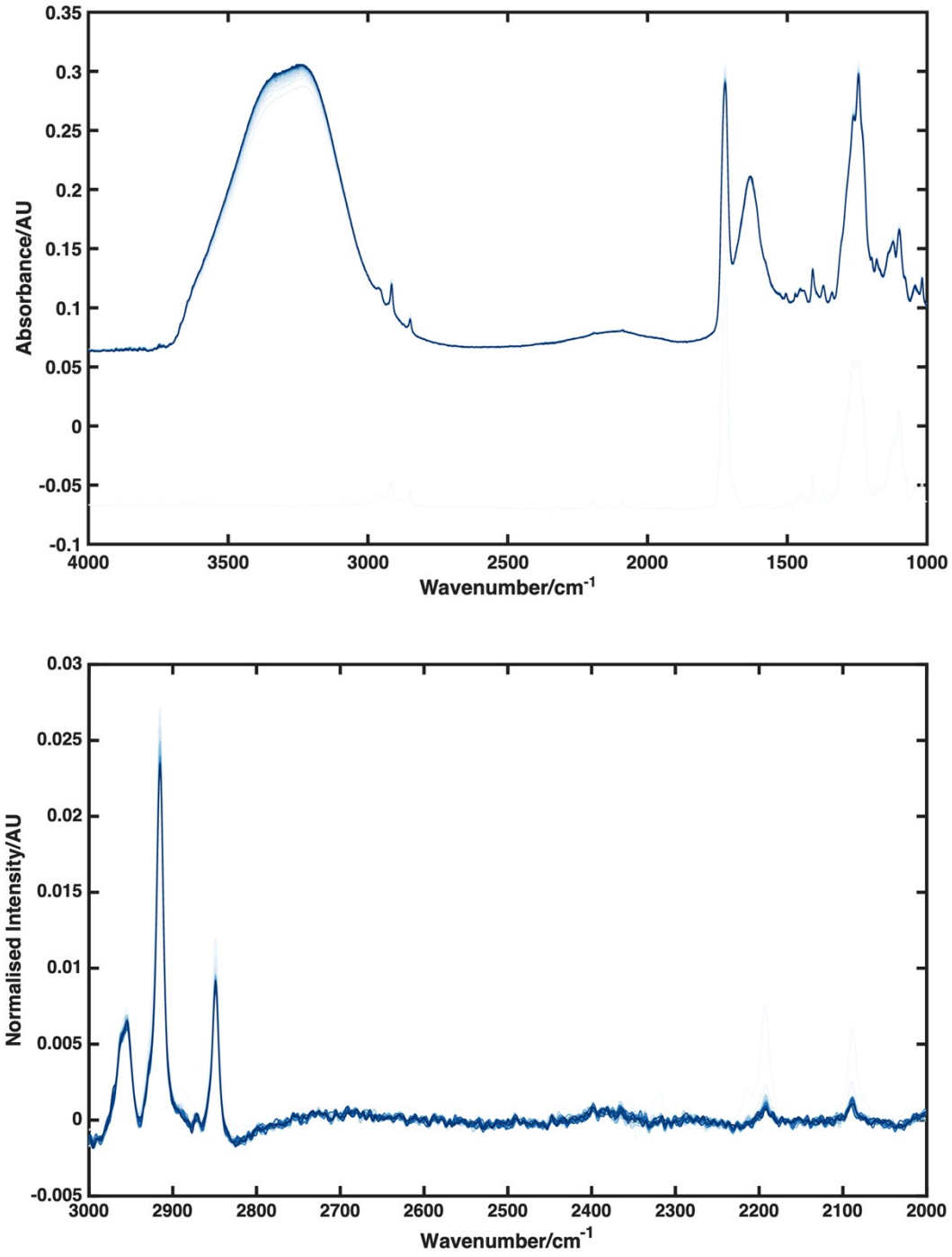


Figure S5: Representative Raw (top) and processed (bottom) spectra for wash-off of tripalmitin and palmitic acid-d using Solution D

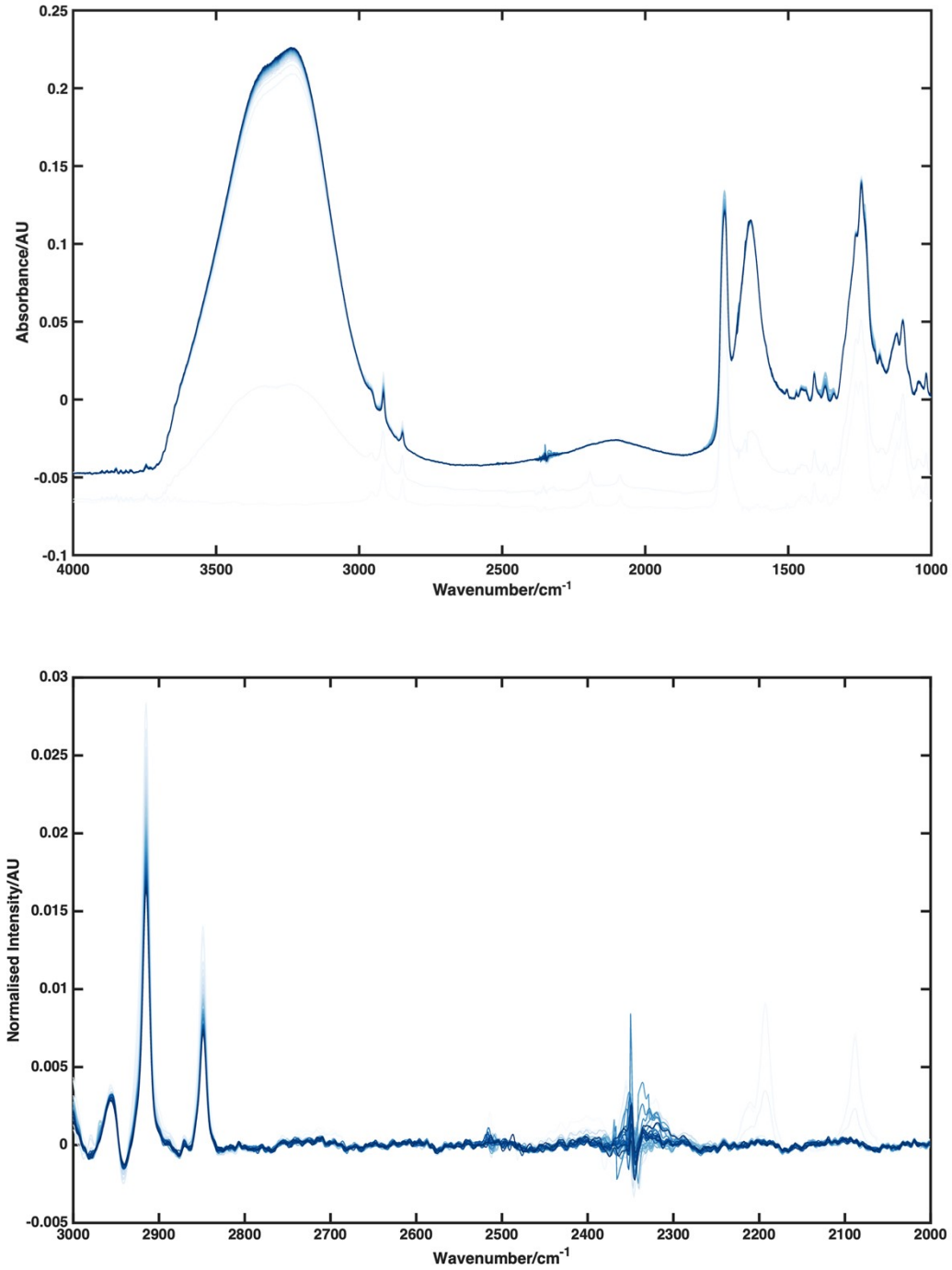


Figure S6: Representative Raw (top) and processed (bottom) spectra for wash-off of tripalmitin and palmitic acid-d using Solution E

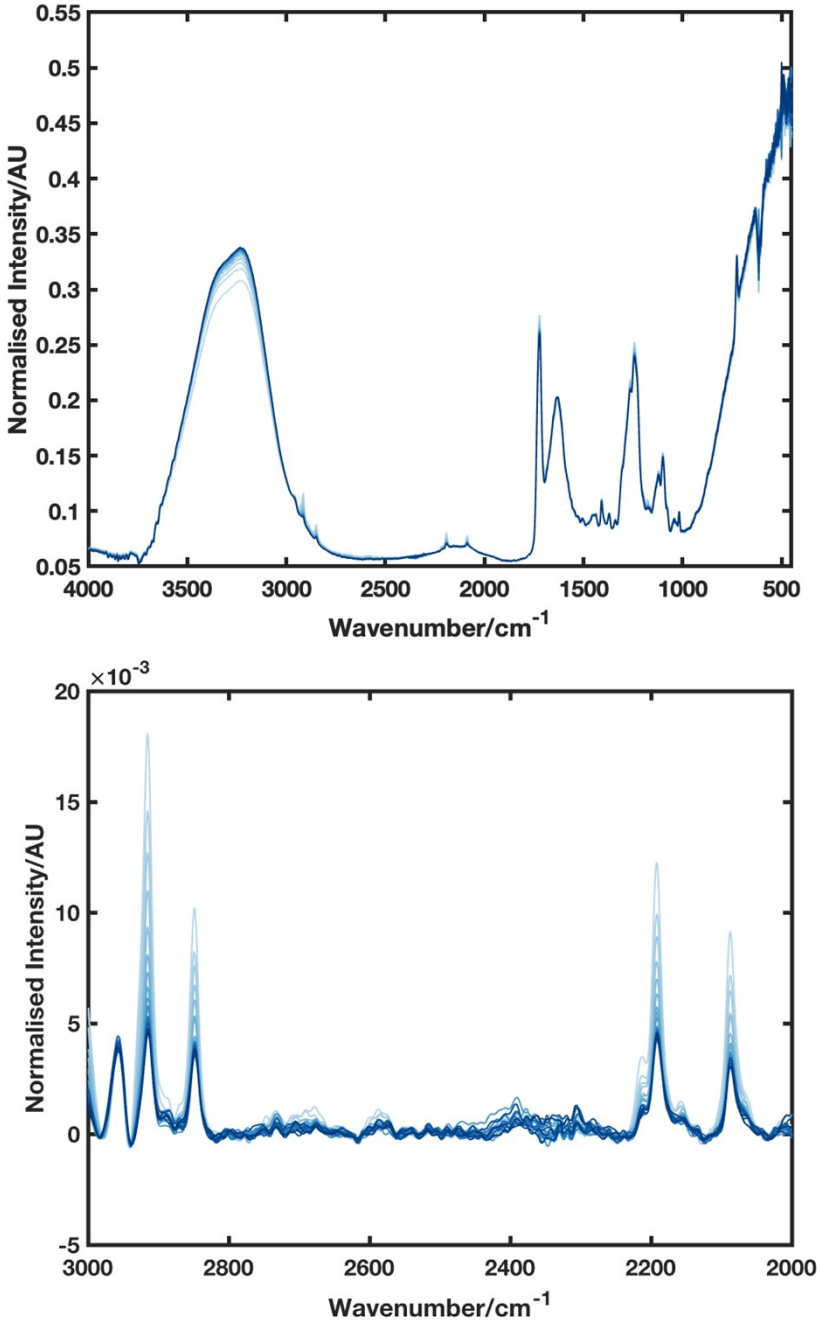


Figure S7: Representative Raw (top) and processed (bottom) spectra for wash-off of tripalmitin and palmitic acid-d using Solution F

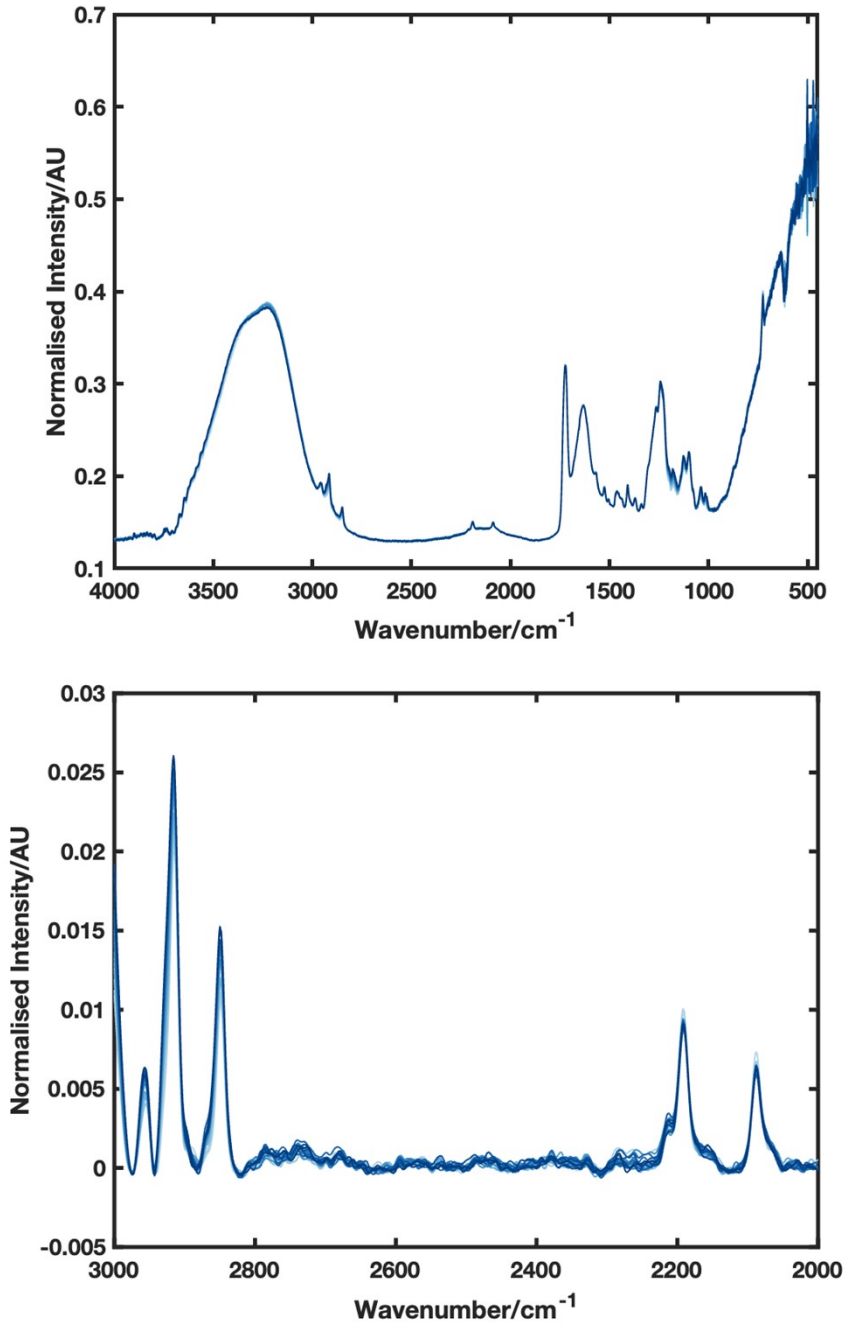


Figure S8: Representative Raw (top) and processed (bottom) spectra for wash-off of tripalmitin and palmitic acid-d using Solution G

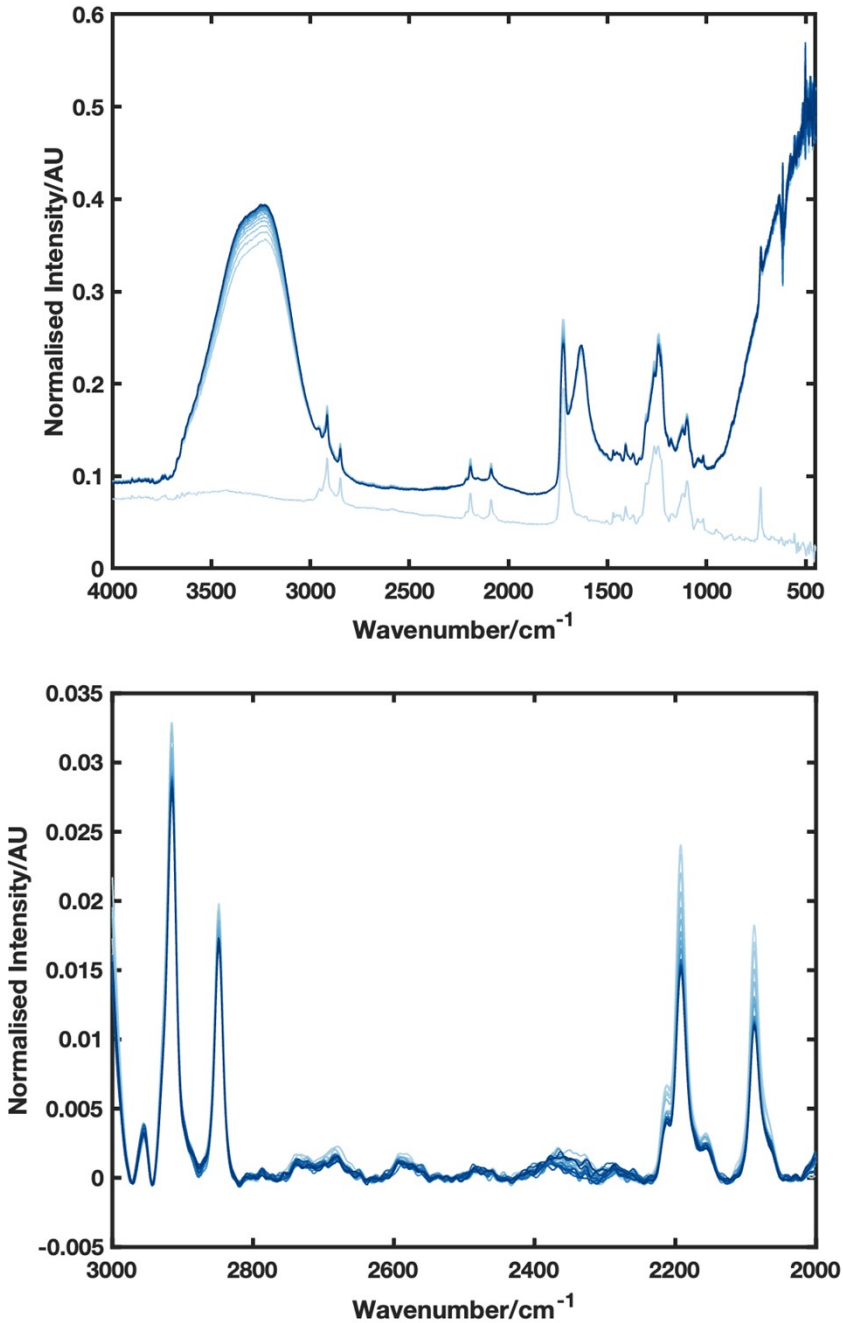


Figure S9: Representative Raw (top) and processed (bottom) spectra for wash-off of tripalmitin and palmitic acid-d using Solution H

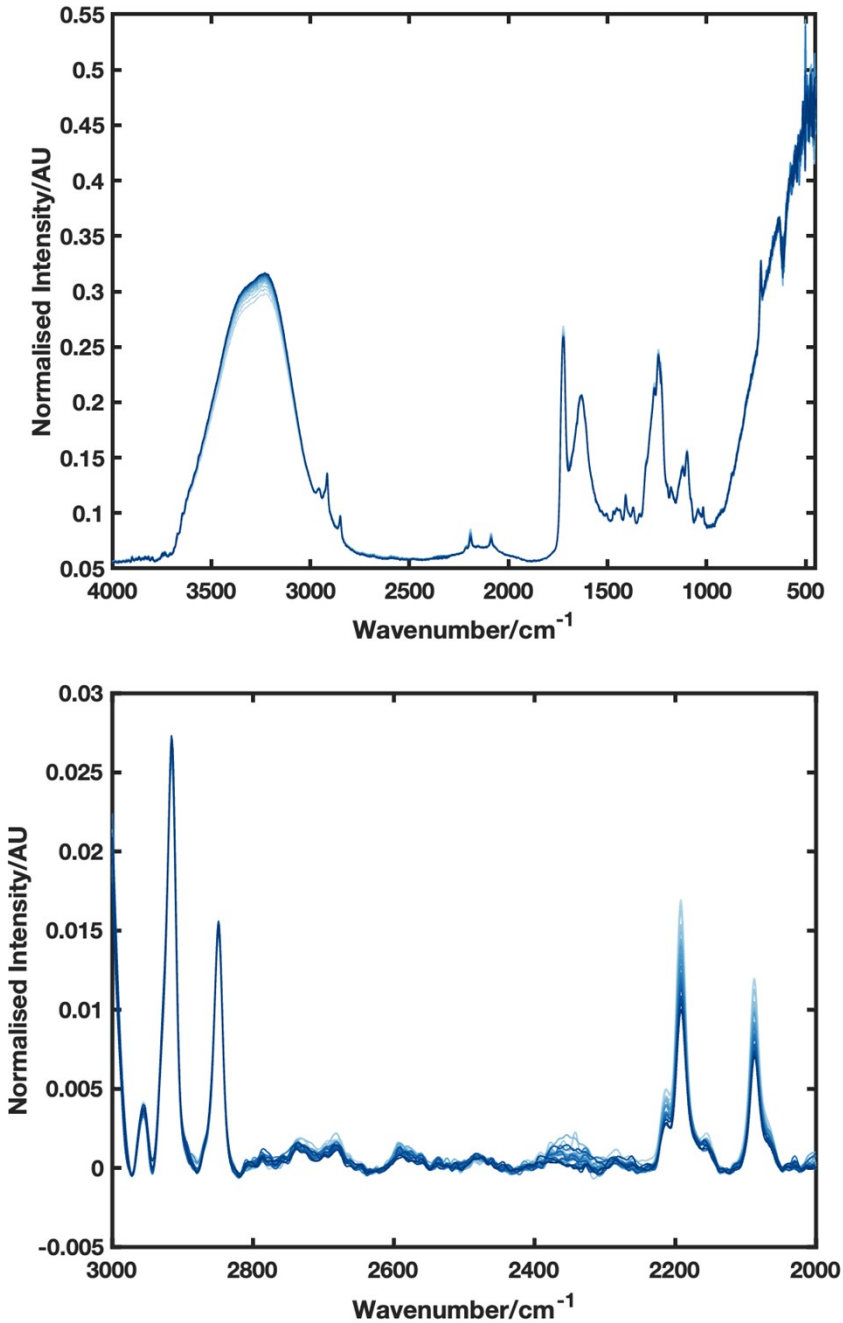


Figure S10: Representative Raw (top) and processed (bottom) spectra for wash-off of tripalmitin and palmitic acid-d using Solution I

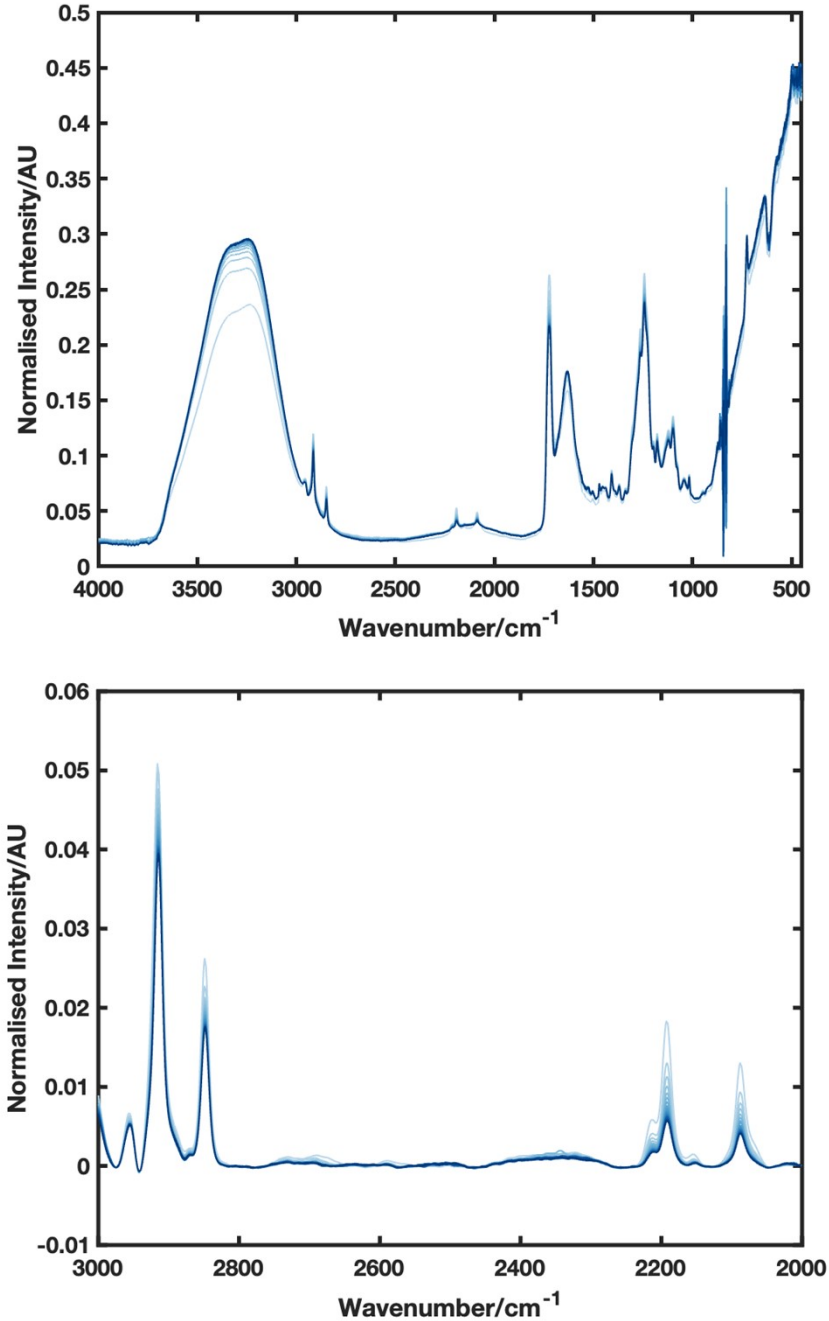


Figure S11: Representative Raw (top) and processed (bottom) spectra for wash-off of tripalmitin and palmitic acid-d using Solution J

Calculation of ATR Penetration Depth

The penetration depth, d_p, of the ATR crystal was calculated using equation S4:

$$d_p = \frac{\lambda}{2\pi n_1(\sin^2\theta - \frac{n_2}{n_1})} \#(S4)$$

Where λ is the wavelength, n_1 and n_2 are the refractive indices of the ATR crystal (Silicon – 3.42)[2] and sample respectively, and θ is the angle of incidence. The structured grooves on the ATR crystal convert the "outer" angle of incidence to an "inner" angle of incidence. The calculation is performed at 26.67 °, which corresponds to an outer angle of incidence of 20 °.

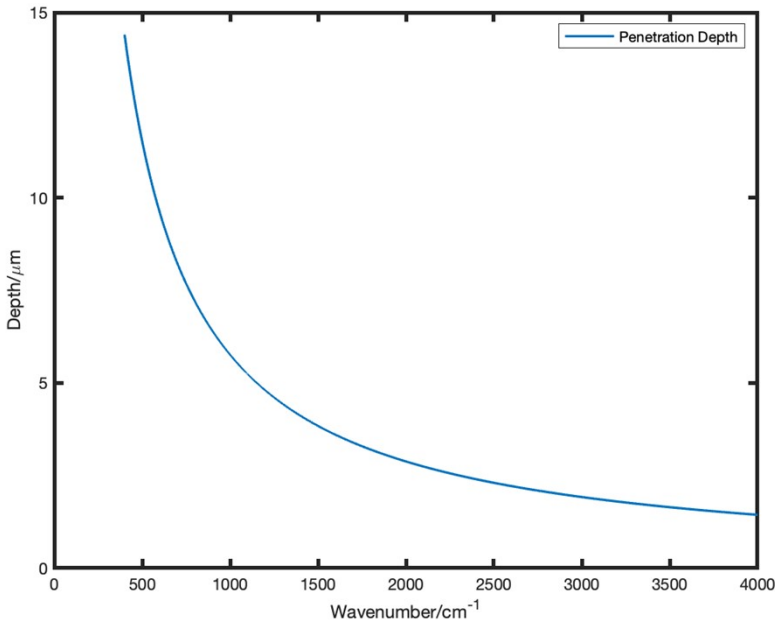


Figure S12: penetration depth as a function of wavenumber for a sample with refractive index 1.5 at an angle of incidence of 26.67 °.*

Signal Intensity Estimation

The intensity of an evanescent wave propagating through a sample is dependent on the wavelength of the incident beam, and the thickness of the sample. It decays exponentially.[3] Equation S2 shows this relation

$$I(z) = I_0 e^{\gamma z} \#(S5)$$

Where γ is the wavelength, z is the distance from the interface, I is the intensity of the evanescent field, and I_0 is the intensity at the sample/ATR crystal interface. The depth of penetration is defined as the height at which the signal intensity is equal to 1/e:

$$d_p = \frac{1}{\gamma} \#(S6)$$

* The manufacturer states that this is the optimal angle of incidence for the Si IRE.

Substituting this relation into S2 gives equation 2:

$$I(z) = I_0 e^{-\frac{z}{d_p}} \#(S7)$$

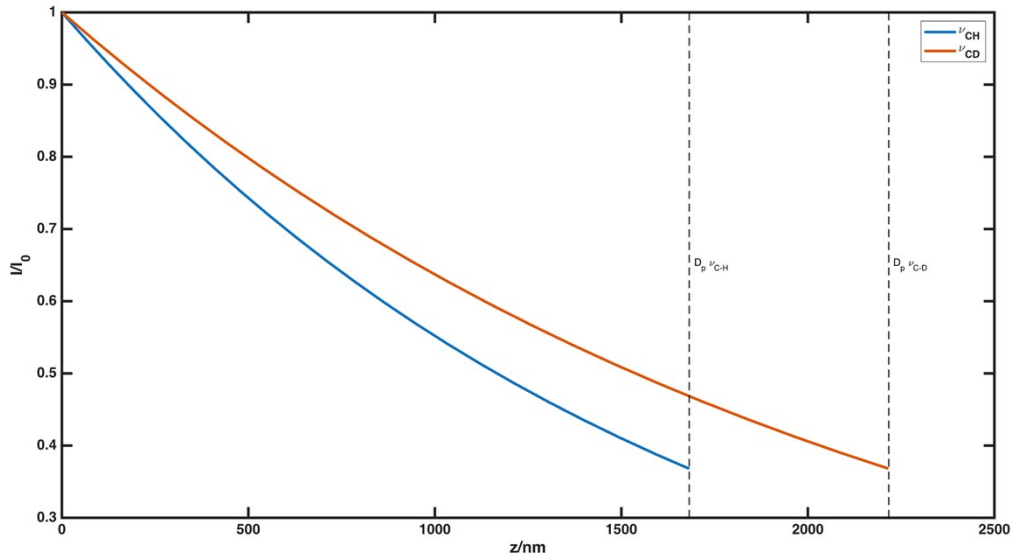


Figure S13: Ratio of I(z) to I_0 as a function of z calculated for C-H and C-D stretches.

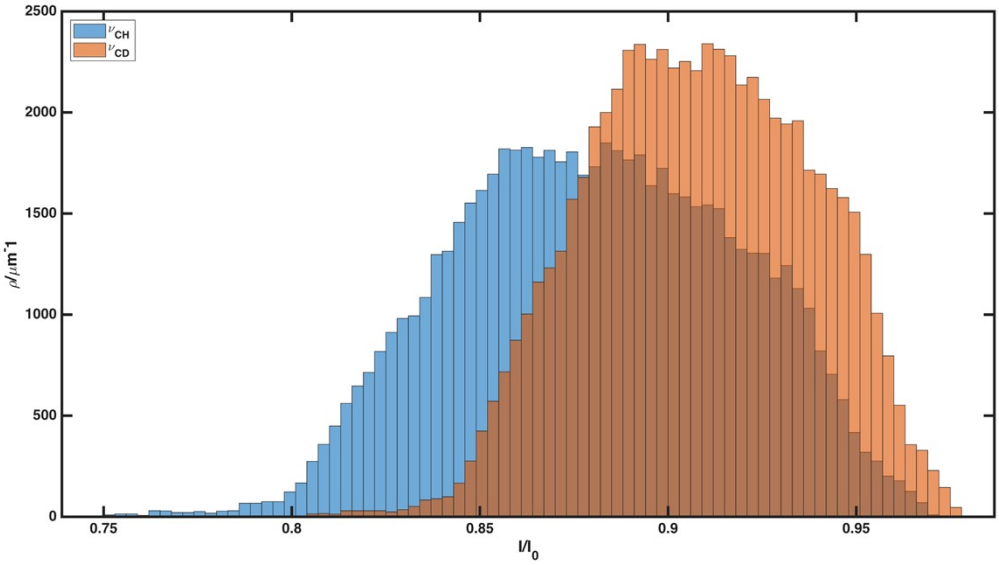


Figure S14: Histogram of estimated intensities from AFM heightmap of unsoiled film.

AFM Determination of Film Height

The thickness of PET and Lipid films were determined by scratching a line into the prepared film using a piece of silicon. AFM images were repeated in three locations across the scratch, the thickness was determined by measuring the height from the bottom of the scratch to the film layer. For the lipid soil, the scratch was used to determine a zero point and the height averaged across the unscratched portion of the image.

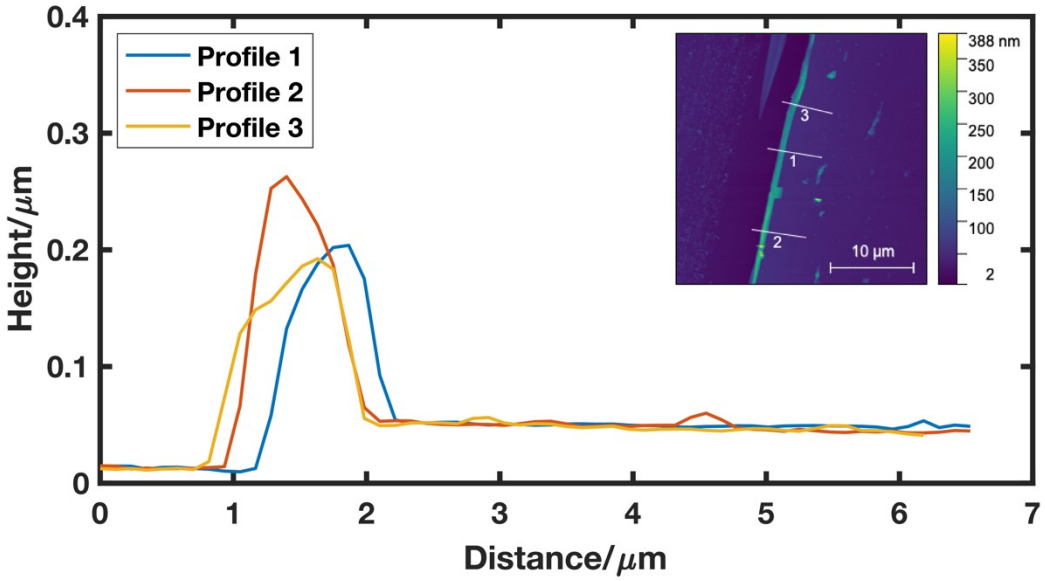


Figure S15: AFM height profile of scratched PET thin film.

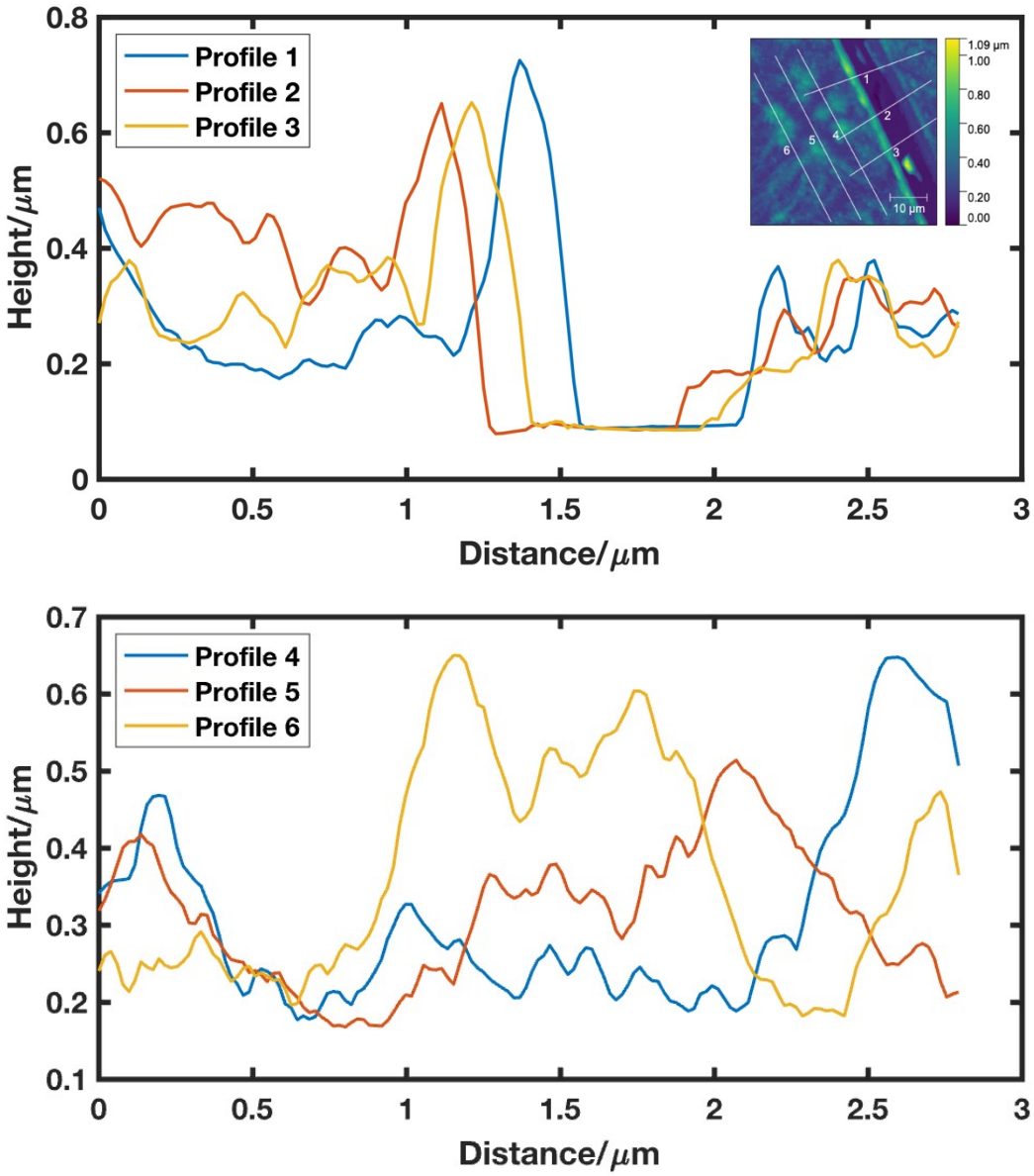
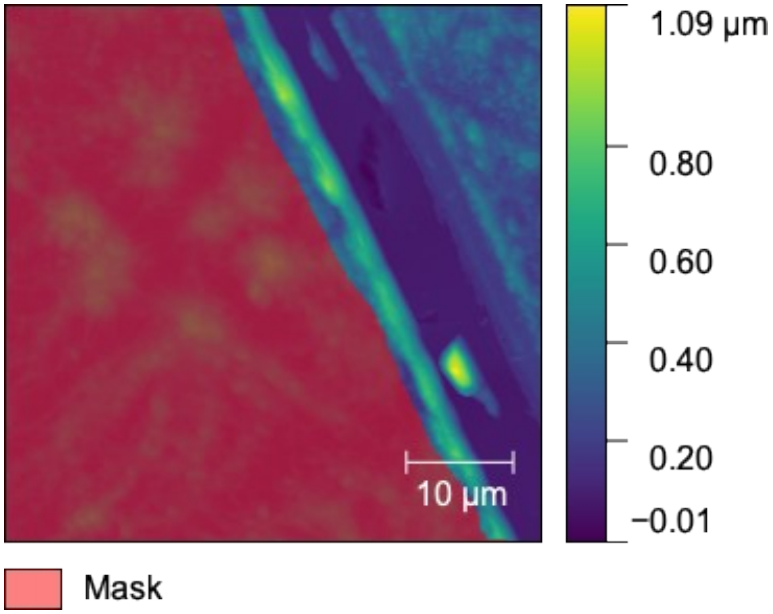


Figure S16: AFM height profiles of scratched lipid soil on PET thin film.



Statistical Quantities
File: /Users/jamesbarclay/Documents/OneDrive - Durham University/PDRA/AFM/Tripalmitin+Palmitic Acid on PET.004
Image: Height
Selected area: 256 × 256 at (0, 0) px
0.000000 × 0.000000 at (0.000000, 0.000000) m
Mask in use: Yes

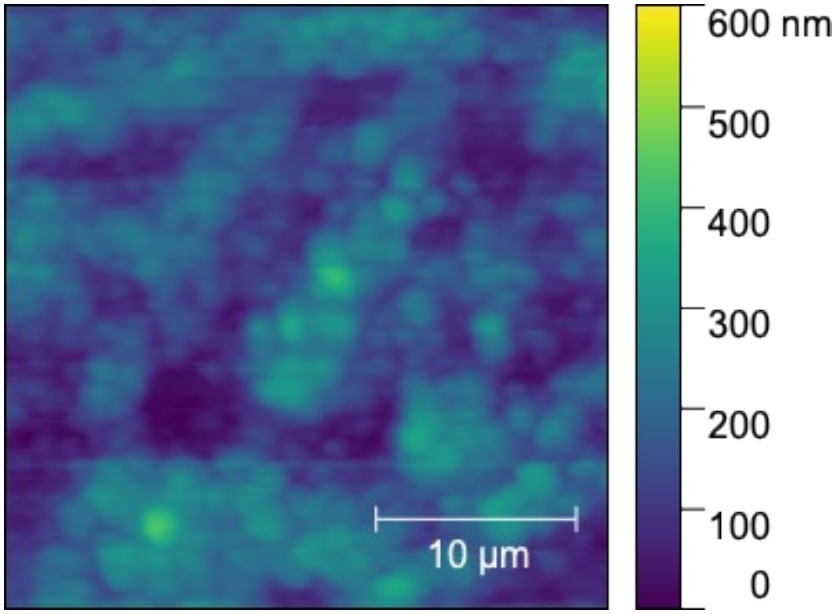
Average value: 280.778 nm
RMS roughness (Sq): 91.5422 nm
RMS (grain-wise): 91.5422 nm
Mean roughness (Sa): 71.1121 nm
Skew (Ssk): 1.24275
Excess kurtosis: 1.46997

Minimum: 146.667 nm
Maximum: 701.564 nm
Median: 257.476 nm
Maximum peak height (Sp): 420.786 nm
Maximum pit depth (Sv): 134.112 nm
Maximum height (Sz): 554.897 nm

Projected area: 1600.61 µm²
Surface area: 1613.71 µm²
Surface slope (Sdq): 0.131546
Volume: 449.432 µm³
Variation: 178.047 µm²

Scan line discrepancy: 0.0317897

Figure S17: Representative AFM image of tripalmitin and palmitic acid spin cast onto a PET substrate. The layer has been scratched to determine film height.



Statistical Quantities
File: /Users/jamesbarclay/Documents/OneDrive - Durham University/PDRA/AFM/Tripalmitin+Palmitic Acid on PET.001
Image: Height
Selected area: 256 × 256 at (0, 0) px
0.000000 × 0.000000 at (0.000000, 0.000000) m
Mask in use: No

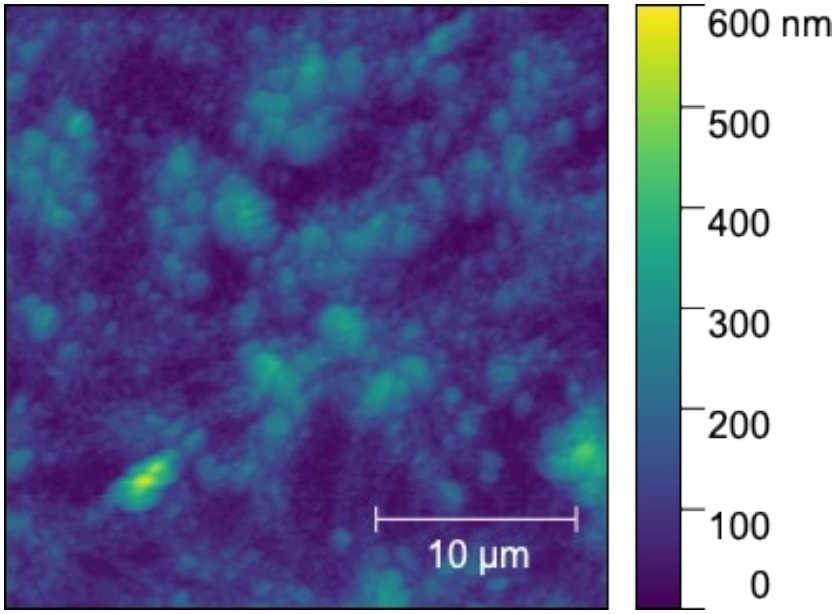
Average value: 165.274 nm
RMS roughness (Sq): 71.9005 nm
RMS (grain-wise): 71.9005 nm
Mean roughness (Sa): 59.5525 nm
Skew (Ssk): 0.165610
Excess kurtosis: -0.513782

Minimum: 0.000 nm
Maximum: 433.620 nm
Median: 163.767 nm
Maximum peak height (Sp): 268.346 nm
Maximum pit depth (Sv): 165.274 nm
Maximum height (Sz): 433.620 nm

Projected area: 900.000 µm²
Surface area: 904.680 µm²
Surface slope (Sdq): 0.103027
Volume: 148.747 µm³
Variation: 77.7233 µm²
Inclination θ: 0.07 deg
Inclination φ: 86.60 deg

Scan line discrepancy: 24.5157×10^{-3}

Figure S18: Representative AFM image of tripalmitin and palmitic acid spin cast onto a PET substrate.



Statistical Quantities
File: /Users/jamesbarclay/Documents/OneDrive - Durham University/PDRA/AFM/Tripalmitin+Palmitic Acid on PET Washoff.003
Image: Height
Selected area: 256 × 256 at (0, 0) px
0.000000 × 0.000000 at (0.000000, 0.000000) m
Mask in use: No

Average value: 110.733 nm
RMS roughness (Sq): 64.7731 nm
RMS (grain-wise): 64.7731 nm
Mean roughness (Sa): 49.7344 nm
Skew (Ssk): 1.32217
Excess kurtosis: 2.70825

Minimum: 0.000 nm
Maximum: 577.342 nm
Median: 96.375 nm
Maximum peak height (Sp): 466.609 nm
Maximum pit depth (Sv): 110.733 nm
Maximum height (Sz): 577.342 nm

Projected area: 900.000 µm²
Surface area: 912.324 µm²
Surface slope (Sdq): 0.171984
Volume: 99.6600 µm³
Variation: 130.689 µm²
Inclination θ: 0.09 deg
Inclination φ: 83.02 deg

Scan line discrepancy: 0.0637839

Figure S19: Representative AFM image of tripalmitin and palmitic acid spin cast onto a PET substrate after washing with surfactant solution A.

PET Solubility Control Experiment

A 25 mm silicon wafer was spin coated with 200 µL 2 wt.% amPET in $CHCl_3$ at 3000 rpm, for 30 s. A transmission IR spectrum was acquired of the wafer. The wafer was then exposed to toluene and spun at 3000 rpm for 30s. A transmission IR spectrum was then acquired of the wafer. Figure S13 shows the two spectra overlaid, showing no loss of intensity.

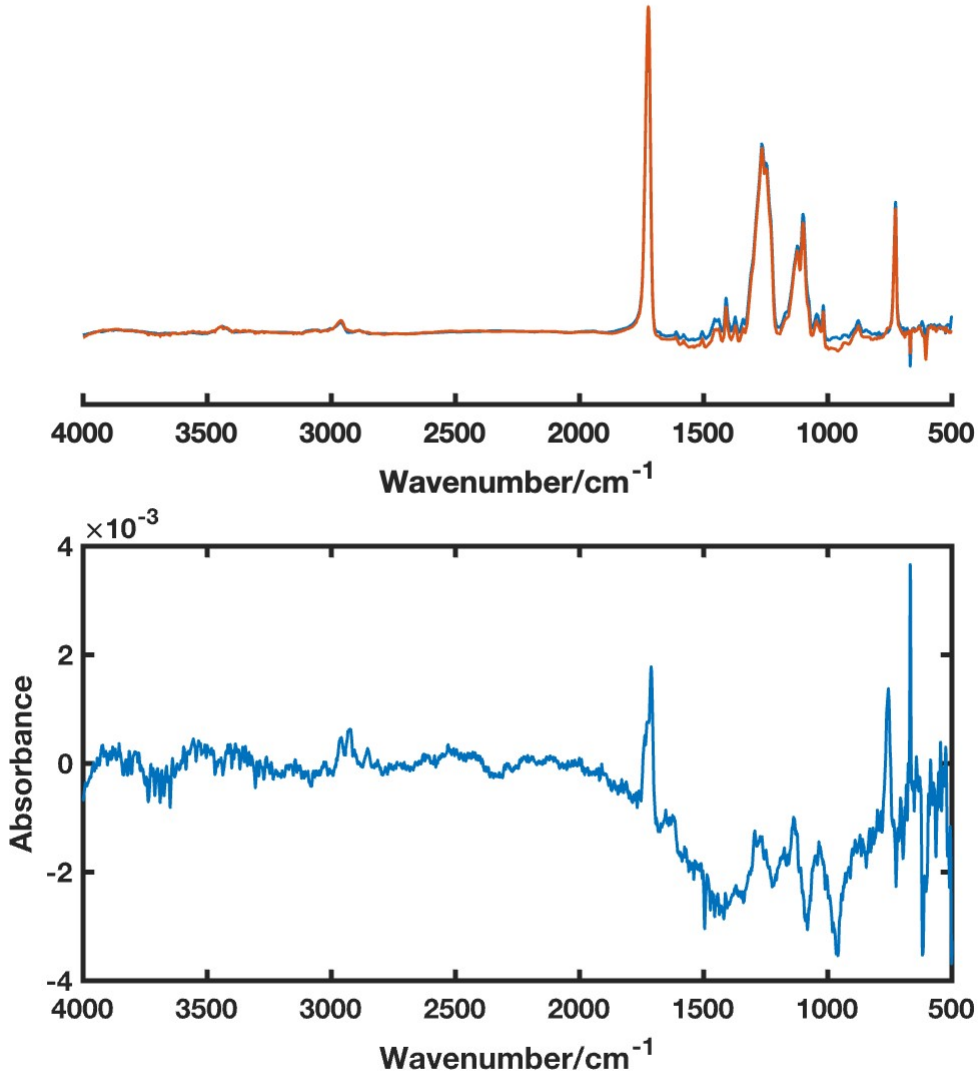


Figure S20: Overlaid (top) before and after exposure to toluene (top) and difference spectrum (bottom).

UNSOILED SAMPLE CONTROL EXPERIMENT

A silicon ATR Crystal was spin coated with 2 wt.% amPET in $CHCl_3$ at 3000 rpm for 30s, then placed in the flow cell and washed with water. This was repeated with surfactant solution a (1:1 LAS/NI, 600 ppm, Solution A) at room temperature

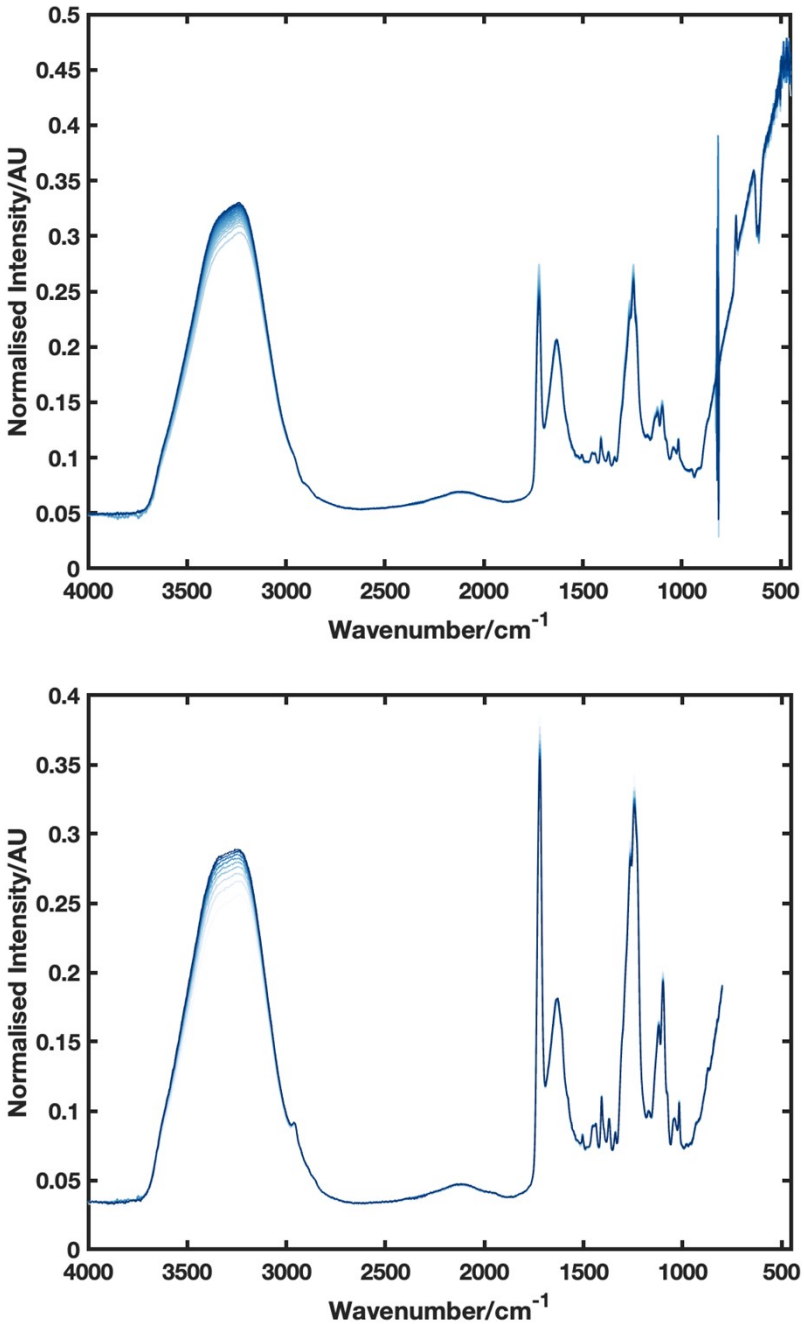


Figure S21: Raw spectra when water (top) and surfactant (Solution A, bottom) is passed over an unsoiled PET thin film.

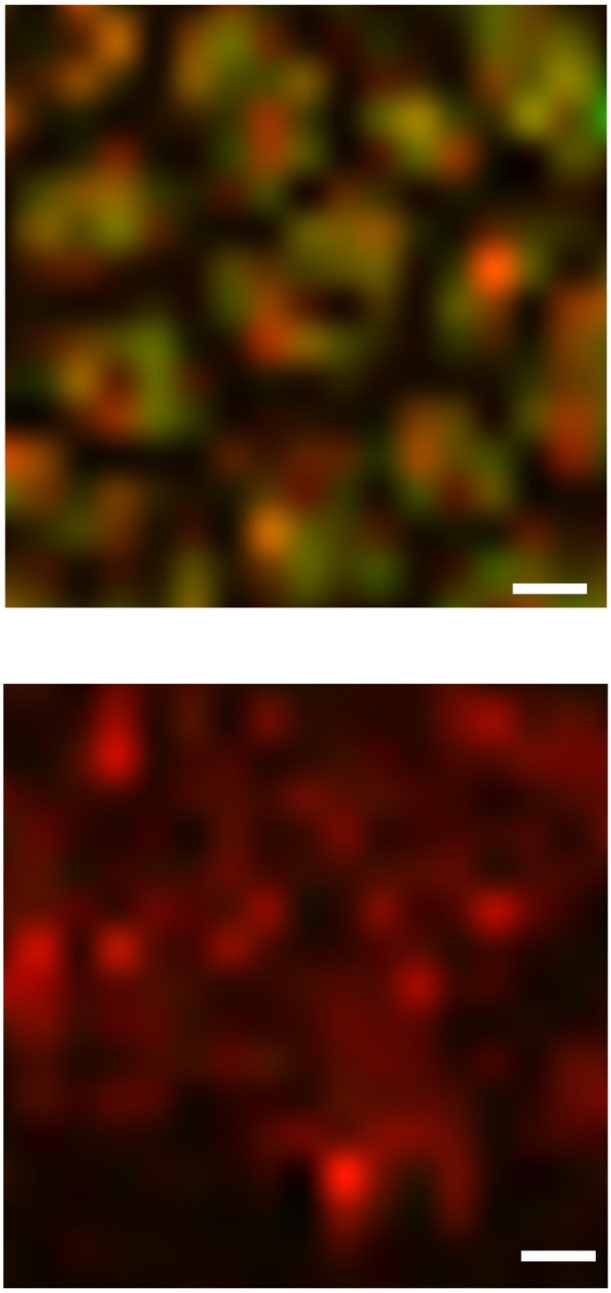


Figure S22: False colour images of $\nu_{C\text{-}H}$ and $\nu_{C\text{-}D}$ bands assigned to the red and green. Channels of respectively before (top) and after washing (bottom), scale bar = 5 µm.

PA-D VS PA-AZ WASH-OFF CONTROL EXPERIMENT

A Silicon ATR crystal was spin coated with 2 wt.% amPET in $ChCl_3$ at 3000 rpm for 30s, followed by a 1:1 mixture of Pa-D and PA-Az (2 wt.% in toluene). The sample was then placed in the flow cell and washed with surfactant solution (1:1 LAS/NI, 600 ppm) for 40 minutes at 25 °C.

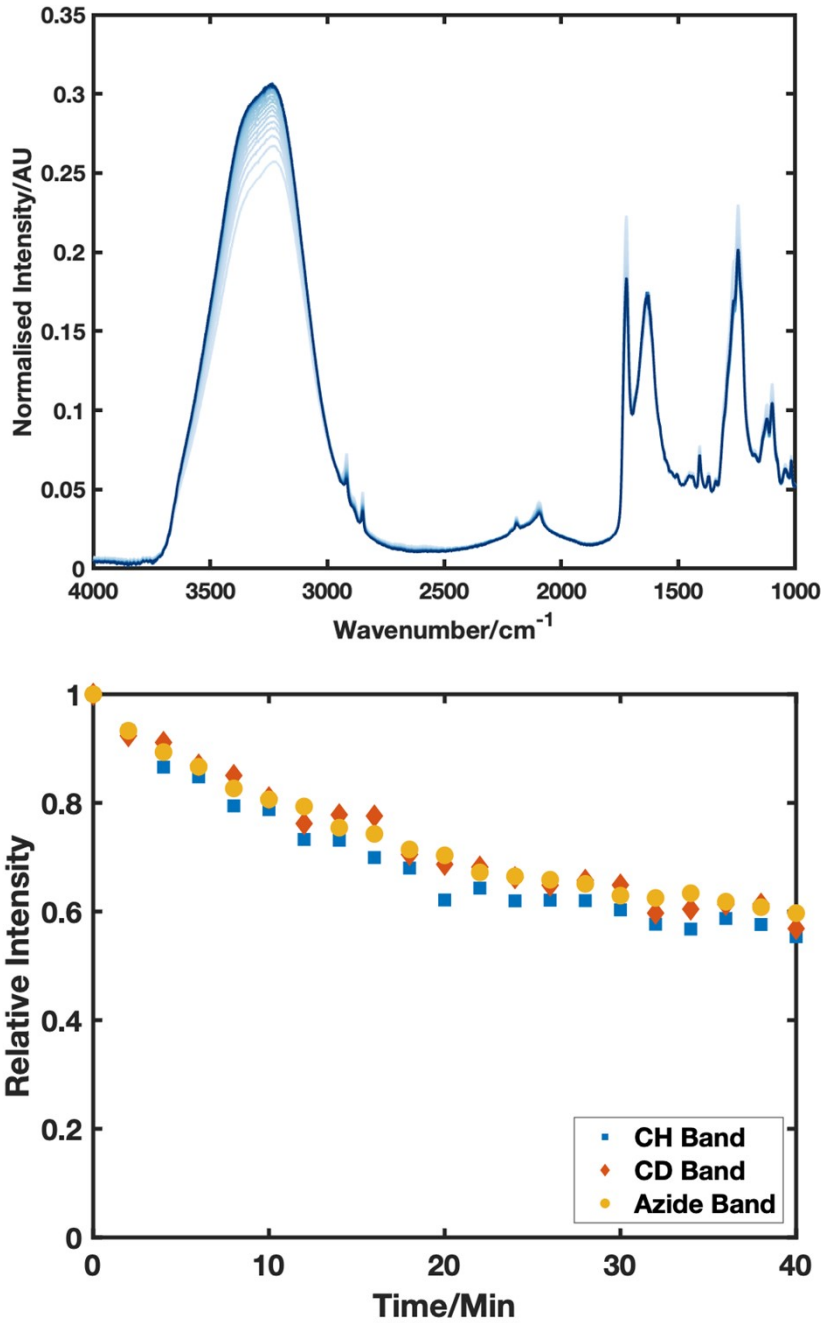


Figure S23: Wash-off curve of a 1:1 mixture of palmitic acid-d and palmitic acid-N_3 under surfactant flow (solution A).

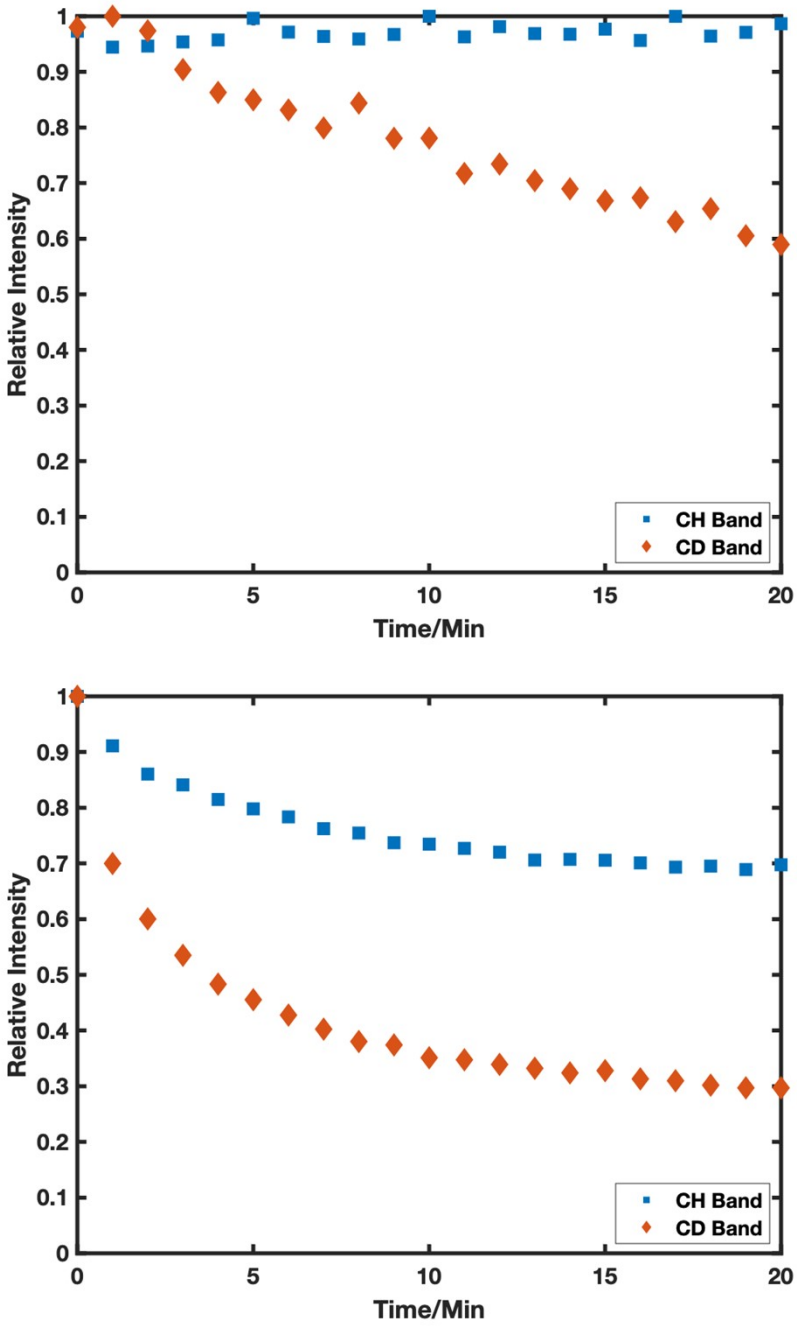


Figure S24: Wash-off curves for LAS/NI (600 ppm) with added NaCl (top, Solution I) and $MgCl_2$ (bottom, Solution J)

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