Electronic Supplementary Information (ESI)

Feasibility of Attenuated Total Reflection – Fourier Transform Infrared (ATR-FTIR) chemical imaging and Partial Least Squares Regression (PLSR) to predict protein adhesion on polymeric surfaces.

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S.1 Fibrinogen standard calibration and quantification of adhesion on polymer film surface using microBCA assay

The microBCA assay uses bicinchoninic acid (BCA) as the detection reagent for Cu^{+1}, which is formed when Cu^{+2} is reduced by protein in an alkaline environment[1]. A purple-coloured reaction product is formed by the chelation of two molecules of BCA with one cuprous ion (Cu^{+1}). This water-soluble complex exhibits a strong absorbance at 562 nm that is linear with increasing protein concentrations. The macromolecular structure of protein, the number of peptide bonds and the presence of four amino acids (cysteine, cystine, tryptophan and tyrosine) are reported to be responsible for colour formation with BCA. Studies with di-, tri- and tetrapeptides suggest that the extent of colour formation is caused by more than the mere sum of individual colour-producing functional groups[2].

Materials and methods

1. Pierce microBCA assay (Catalog number: 23235, 3 reagent version)

2. Bovine Fibrinogen (CAS Number 9001-32-5)

3. Corning 'orange cap' polypropylene 50ml centrifuge tube (20 tubes) (Catalogue No 10604551)

4. Saline (Weigh dessicator stored 9g NaCl, into 1L beaker, fill upto 1L with deionised water).

5. Phosphate Buffer Solution 0.1M (1X) (PBS) (CAS Number AM9625, pour 100ml PBS (stock 10X) into 1L beaker, fill upto 1L with DI water.)

6. Sodium Dodecyl Sulphate (SDS) in PBS (1% SDS by weight) (CAS Number H5115, Weigh 1g SDS, place into 1L beaker, fill upto 1L using 10X PBS).

7. 100-1000ul pipettes (Catalogue No 12360613) and holder.

8. Glass test tubes (36 test tubes, wash with detergent, deionised water, acetone dried)

9. UV-Spectrophotometer warmed up to 1 hour prior to measurements equipped with quartz cuvettes.

10. Magnetic stirrer/heater equipped with temperature monitoring.

Preparation of diluted fibrinogen (Fb) standards and working reagents

1. Weigh 0.02 g of fibrinogen on the mass balance and place in beaker 1. Add a magnetic stirrer to this beaker.

2. Pre-warm saline (37 ºC) and pour into beaker 1 upto 20 ml. Magnetically stir at 125rpm, 37 ºC for 1 hour till solution turns hazy.

3. Pour solution into 50ml tube and mark as 'S1: 1000 µg /ml'.

4. Measure 10ml of S1, pour into beaker 2 and fill to 100ml using PBS, mark as 'S2: 100 µg/ml'.

5. Prepare dilutions as follows: -

Table S.1 Preparation of diluted bovine fibrinogen standards

Tube	Final Fb concentration	take (ml)	from	add (PBS/SDS) ml	Total (ml)	Remaining (ml)
F1	100µg/mL	5	S2	0	8	4.8
F2	40µg/mL	3.2	F1	4.8	8	4
F3	20µg/mL	4	F2	4	8	2
F4	15ug/ml	6	F3	2	8	2.667
F5	10µg/mL	5.333	F4	2.667	8	4
F6	5µg/mL	4	F5	4	8	4
F7	2.5µg/mL	4	F6	4	8	4.8
F8	1µg/mL	3.2	F7	4.8	8	4
F9	0.5µg/mL	4	F8	4	8	8
F10	0µg/mL = Blank	0	**0**	8	8	5

6. Prepare working reagent by mixing 25 parts of microBCA reagent MA and 24 parts reagent MB with 1 part of reagent MC. For example, for 30 test samples, mix 15ml reagent MA, 14.4 ml reagent MB and 0.6 ml of reagent MC in a 50 ml polypropylene tube. A fresh solution gives a green coloured solution, which can be used for upto 24 hours kept at room temperature.

Test tube procedure (linear working range of 0.5-20µg/mL)

1. Pipette 1.0 mL of each standard and unknown sample replicate into appropriately labelled glass test tubes.

2. Add 1.0 mL of the working reagent to each tube and mix well.

3. Cover tubes and incubate at 60°C in a water bath for 1 hour.

4. Cool all tubes to room temperature.

5. With the spectrophotometer set to 562 nm, zero the instrument on a cuvette filled only with water. Subsequently, measure 3 values of absorbance of all the samples within 10 minutes.

Note: - Colour development continues even after cooling to room temperature. However, the rate of development at RT is sufficient low that no significant error is introduced if all absorbance measurements are made within a 10-minute period.

7. Prepare a standard curve by plotting the average 562nm reading for each BSA standard vs. its concentration in µg/ml. Use the standard curve to determine the protein concentration of each unknown sample.

S.2 Wet regression vector and mean wet spectra of test polymeric surfaces

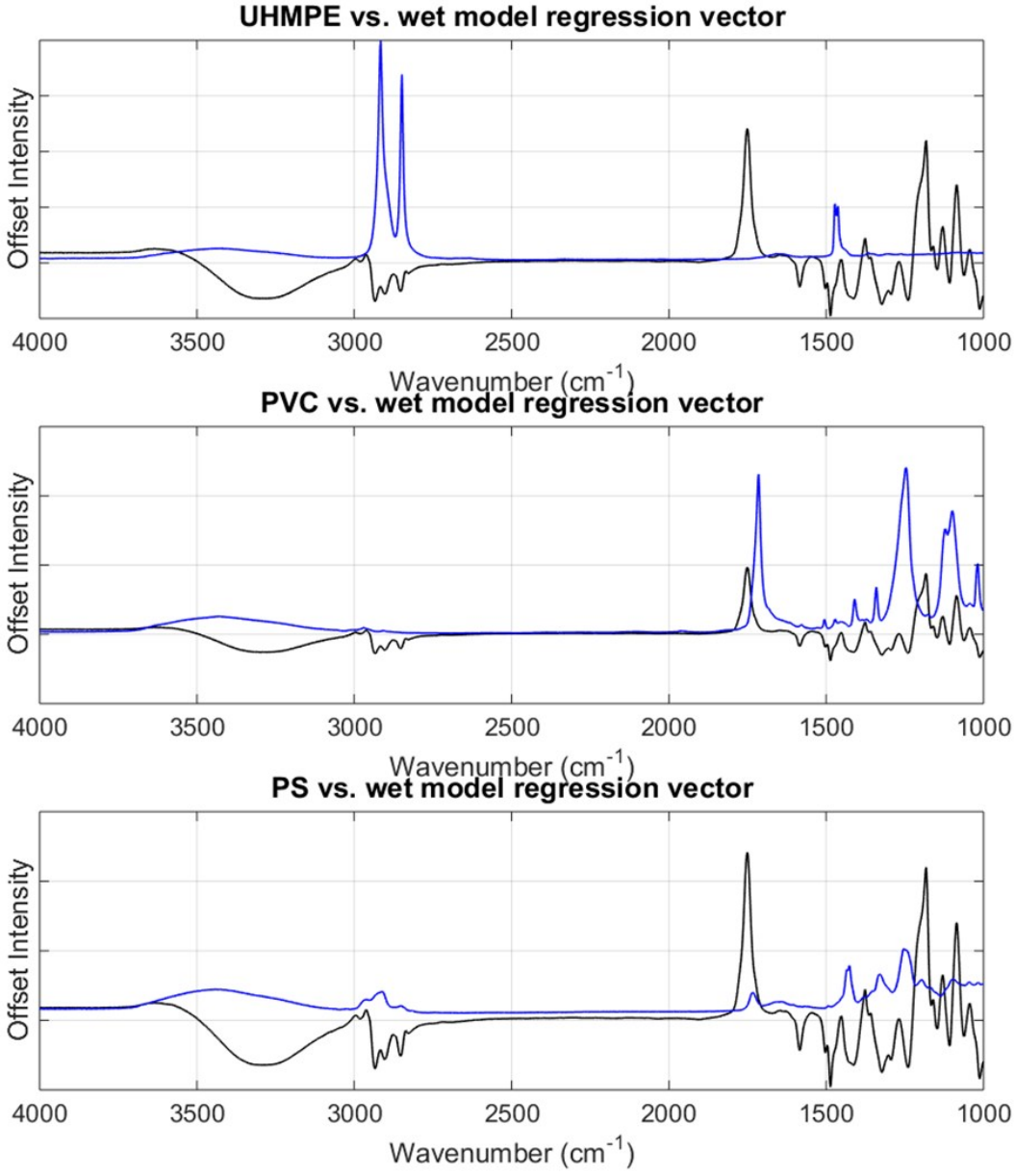

Figure S.1 Comparison of the spectral features of the regression vector (in black) from the best wet model and the wet infrared spectra (in blue) of the test/validation polymers (UHMPE, PVC, PS).

References

1. Smith, P. K. *et al.* Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150,** 76–85 (1985).

2. Wiechelman, K. J., Braun, R. D. & Fitzpatrick, J. D. Investigation of the bicinchoninic acid protein assay: Identification of the groups responsible for color formation. *Anal. Biochem.* **175,** 231–237 (1988).