Ultra-trace Detection of Human Erythropoetin Using Functionalised-Surface Enhanced Raman Spectroscopy (SERS)

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Abstract

Raman spectroscopy has recently achieved considerable interest for biomedical diagnosis and promising and innovative therapies.[1] The emergence of compact laser sources, more sensitive detection equipment and portable instrumentation have all contributed to this interest. Here we report an ultrasensitive method for detecting bio-active compounds in biological samples by means of functionalized nanoparticles interrogated by surface enhanced Raman spectroscopy (SERS). This method is applicable to the recovery and detection of many diagnostically important peptidyl analytes such as insulin, human growth hormone (somatotropin), mitogenic polypeptide growth factors (IGFs) and erythropoietin (EPO), as well as many small molecule analytes and metabolites. Our method was developed using the EPO system to demonstrate its utility in a complex yet well-defined biological system. Recombinant human EPO (rHuEPO) and EPO analogues have successfully been used to treat anaemia in end-stage renal failure, chronic disorders and infections, cancer and AIDS.[2] Current methods for EPO testing are lengthy, laborious and relatively insensitive to low concentrations.

References

[1] Pahlow, S.; März, A.; Seise, B.; Hartmann, K.; Freitag, I.; Kämmer, E.; Böhme, R.; Deckert, V.; Weber, K.; Cialla, D.; Popp, J. *Eng. Life Sci.* **12** (2012) 131.

[2] Fisher, J. W. *Experimental Biology and Medicine* **228** (2003) 1.

[3] Lonnberg, M.; Drevin, M.; Carlsson, J. *J. Immunol. Methods* **339** (2008) 236.

Figures

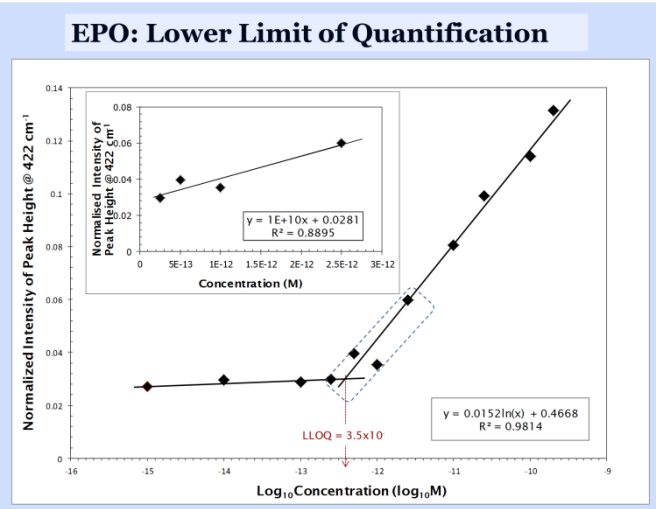

Figure 1: Log_{10} Calibration curve using Area Normalised Peak Height at 422 cm^{-1}, Log_{10} Calibration Range from $2x10^{-10}$M to Lower Limit of Quantification.(LLOQ) = $3.5x10^{-13}$M. Insert linear calibration range $2.5X10^{-12}$M to 2.5 x 10^{-13}M, corresponding to blue dotted box.

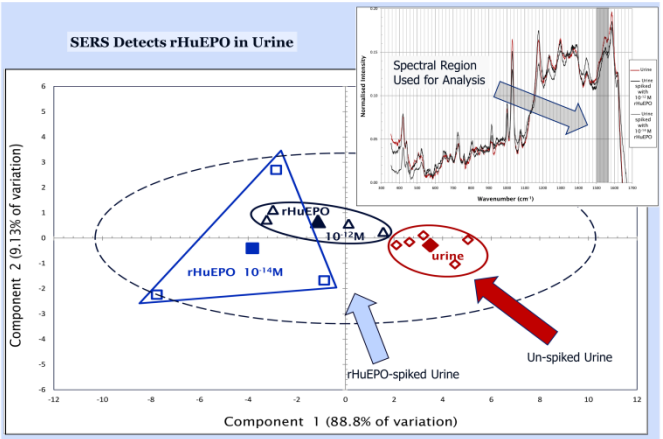

Figure 2: PCA of Raman spectra (1515 -1580 cm^{-1}). All urine samples are diluted 1:1. The larger data points are the average of six measurements. They are well separated even though they each contain measurements that are outliers to the model. Urine with: PBS pH 7.4, rHuEPO at $1x10^{-12}$M in PBS and rHuEPO at $1x10^{-14}$M in PBS respectively. Insert: spectrum from Raman with shaded box showing region used for chemometrics.

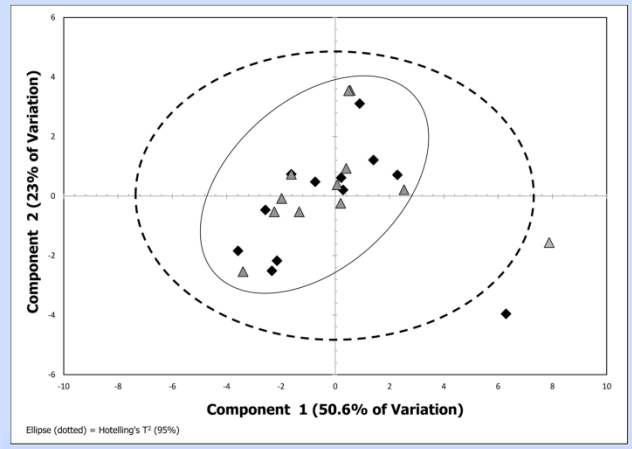

Figure 3: Hierarchal PCA of Raman spectra (354 -1627 cm^{-1}). Black diamonds are unreacted nanoparticles, grey triangles are nanoparticles reacted with skim milk.