Ultra-trace Detection of Diagnostically Important Biomarkers Using Functionalised-Surface Enhanced Raman Spectroscopy (SERS)

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Introduction

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.

EPO: Lower Limit of Quantification

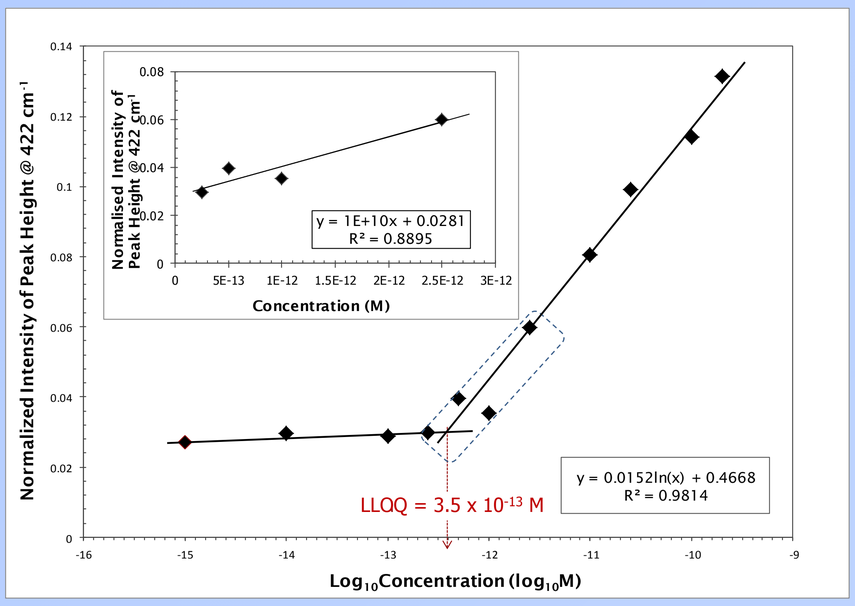

Figure 1: Log_{10} Calibration curve using Area Normalised Peak Height at 422 cm^{-1}, Log_{10} Calibration Range from $2x10^{-10}$M to Lower Limit of Quantification.(LLOQ) = $3.5x10^{-13}$M. Insert linear calibration range $2.5X10^{-12}$M to 2.5 x 10^{-13}M, corresponding to blue dotted box.

SERS Detects rHuEPO in Urine

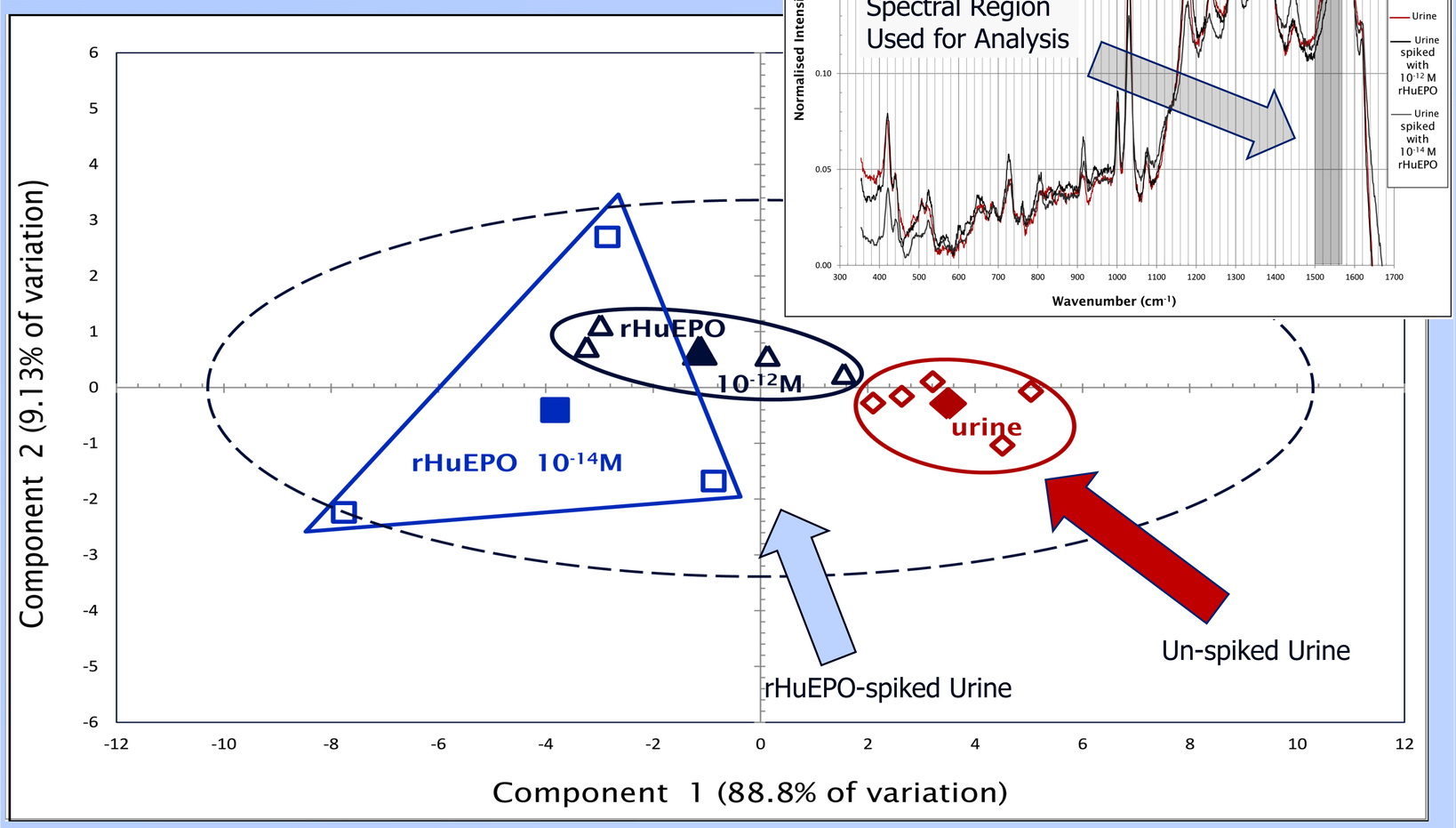

Figure 2: PCA of Raman spectra (1515 -1580 cm^{-1}). All urine samples are diluted 1:1. The larger data points are the average of six measurements. They are well separated even though they each contain measurements that are outliers to the model. Urine with: PBS pH 7.4, rHuEPO at $1x10^{-12}$M in PBS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

Methods

Gold nanoparticles functionalised with anti-EPO antibody (3F6, MAIIA diagnostics) were used to:

- Optimise the conditions for the interaction between the nanoparticle EPO.
- Determine the major Raman peaks of the nanoparticle before and after reaction with EPO.
- Develop a calibration curve for EPO.
- Determine the ability of the nanoparticle to isolate EPO from urine,
- Determine the ability of the Raman technique to distinguish between the EPO and rHuEPO.
- Confirm that the nanoparticle do not cross react nonspecifically with other proteins in biological fluids.

For the Raman studies, 10 µL each of the nanoparticle solution and analyte were mixed; pre-reacted and dried on a microscope slide covered with aluminum foil. When using biological fluids, the reacted nanoparticles were washed several times by centrifugation and resuspension before loading on the microscope slide. Samples were then examined using a Renishaw in Via Raman Microscope with 785 nm laser. The Raman spectra were processed if necessary using Principal Component Analysis (PCA).

Results

The optimal time to react the nanoparticle with EPO was found to be 30 minutes. The limit of quantification was determined to be $3.5x10^{-13}$ M and a log_{10} calibration curve to 2×10^{-10} M was obtained (R^2 = 0.98). This contained a linear calibration region from $2.5x10^{-12}$ M to $2.5x10^{-13}$ M (R^2 = 0.89). See figure 1.
Urine varied from recombinant EPO spiked urine in region:1515 -1580 cm^{-1}. See figure 2, and insert graph

Discussion

Our rapid screening methodology utilised commercially available anti-EPO antibodies to provide very high selectivity towards EPO protein in urine. These "smart sensors" interact with, trap and detect EPO. SERS allows for ultra-trace detection ($<<10^{-15}$ M) and quantification of EPO in biological fluids containing the normal concentration range of human endogenous EPO, e.g. urine and blood serum, [3] (Fig. 1), with minimal sample preparation.

SERS detection differentiated between human endogenous EPO and rhEPO in un-purified urine (Fig 2). The elimination of sample preparation allows direct screening in biological fluids, significantly reducing the time required by current methods. Antibody recognition against a variety of biological targets and the availability of portable commercial SERS analysers for rapid onsite testing provide broad diagnostic applicability in a robust analytical platform.

SERS negative control for cross reactivity

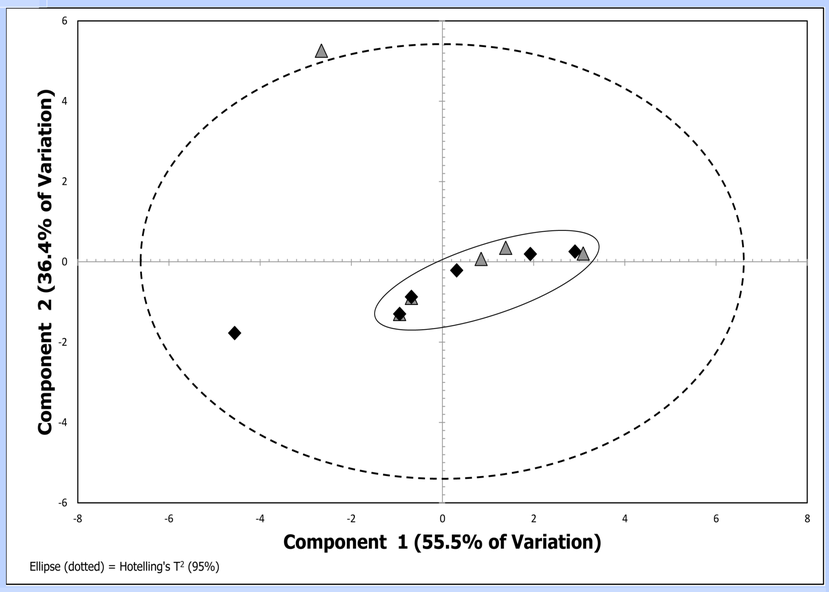

Figure 3: Hierarchal PCA of Raman spectra (354 -1627 cm^{-1}). Grey triangles are unreacted nanoparticles, black diamonds are nanoparticles reacted with skim milk.

References

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(2) Fisher, J. W. *Experimental Biology and Medicine* **2003**, *228*, 1.
(3) Lonnberg, M.; Drevin, M.; Carlsson, J. *J. Immunol. Methods* **2008**, *339*, 236.