

Towards quantitatively reproducible substrates for SERRS - Supplementary Information

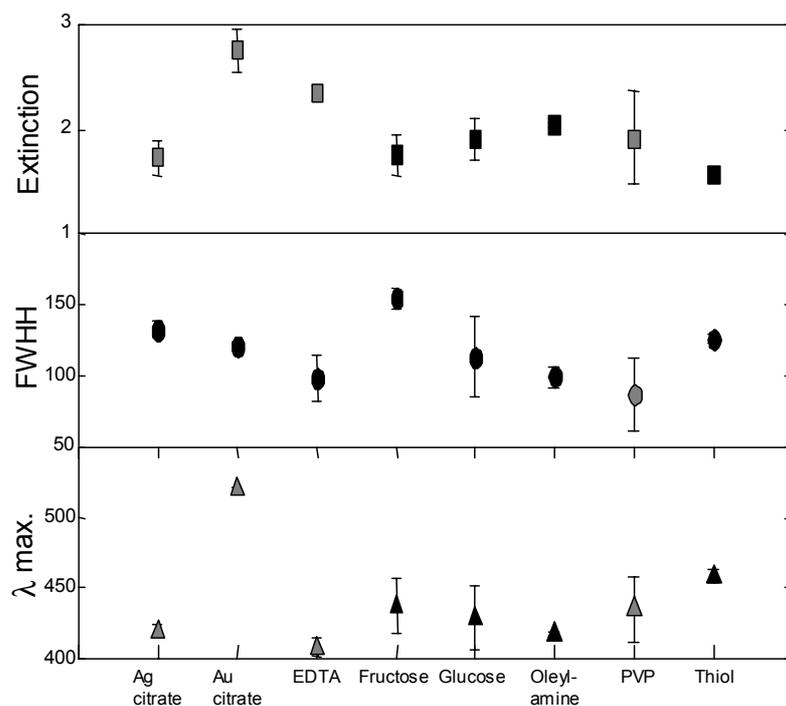
Roger M. Jarvis, Helen E. Johnson, Emma Olembe, Arunkumar Panneerselvam,
Mohammad A. Malik, Mohammad Afzaal, Paul O'Brien and Royston Goodacre

Preparation of Colloids

The sols were prepared according to the following published protocols. For the citrate reduced colloidal silver and gold sols the Lee & Meisel method¹ was used. EDTA reduced colloidal silver was prepared as described by Fabrikanos *et al.*². Fructose and glucose reduced colloidal silver solutions were prepared according to Pal *et al.*³. Oleylamine reduced colloidal silver was prepared by the Hiramatsu and Osterloh method⁴. PVP capped colloidal silver sols were prepared using the method developed by Sun and Xia⁵. Finally, thiol capped colloidal silver was prepared according to Korgel and Fitzmaurice⁶.

Absorption Spectroscopy of Colloids

The absorbance data depicted in Supplementary Figure 1 do not give any clear indication as to why only these four sols (citrate reduced silver and gold, PVP capped silver and EDTA reduced silver) facilitated a SERRS response, as there is no characteristic trend which separates these colloids from those which did not give SERRS.



Supplementary Figure 1. Mean and standard deviation error bars for absorbance measurements taken from 3 independent batch preparations of 8 sols that were subsequently assessed for SERRS activity. λ (absorption) maximum, a larger value equates to a larger particle size. FWHH (full width at half height), a larger FWHH indicates wider particle size distribution. Extinction, a lower value for the extinction indicates greater aggregation⁷

Signal Enhancement

Supplementary Table 1 lists the signal-to-noise ratios (S/N) for the median spectra of the SERRS bands subsequently used in MANOVA (those marked by asterisk in Figure 1, main article), since this metric is of interest to those attempting to optimise a SERRS experiment. Whilst any differences in the intensity of SERRS signal in the enhanced spectra for different substrates are fairly small, the S/N for both citrate reduced and PVP capped colloidal silver are greater than those for both citrate reduced gold and EDTA reduced silver.

Supplementary Table 1. Signal-to-noise ratios (S/N) observed in the median SERRS spectra of cresyl violet for the vibrational bands used in MANOVA.

	877 cm ⁻¹	1049 cm ⁻¹	1186 cm ⁻¹	1277 cm ⁻¹	\bar{x}
Au citrate	1.24	1.20	1.58	1.88	1.47
	846 cm ⁻¹	877 cm ⁻¹	985 cm ⁻¹	1277 cm ⁻¹	
Ag citrate	1.23	1.28	1.81	2.10	1.60
EDTA	1.09	1.11	1.44	1.72	1.34
PVP	1.29	1.28	1.93	2.03	1.63

Multivariate Analysis of Variance (MANOVA)

Multivariate analysis of variance (MANOVA^{8, 9}) uses exactly the same principle as ANOVA, except the data comprise p variates instead of a single variate. Hence, the sums of squares (SS) are replaced by sums of squares and products matrices (SSPM) for the p variates. A comparison between ANOVA and MANOVA can be seen in Supplementary Tables 2(a) and 2(b), which show a generalised form for a one-way experiment in both cases.

Supplementary Table 2(a) Adapted from ¹⁰. An ANOVA table for a one-way situation, where there are t treatments each replicated n times. The other symbols (M and s^2) are as defined in the table, F is the test statistic.

	Sum of squares	Degrees of freedom	Mean square	F
Treatment	$(t-1)M$	$(t-1)$	M	$\frac{M}{s^2}$
Residual	$t(n-1)s^2$	$t(n-1)$	s^2	-
TOTAL	Sum of above	$tn-1$	-	-

Supplementary Table 2(b). A MANOVA table for a one-way situation, where there are t treatments each replicated n times. The square matrices H , R and T are $p \times p$ in dimension, where p is the number of variates involved. F is the test statistic approximation calculated from Wilks' Λ -Lambda (Λ).

	Sum of squares and products matrices	Degrees of freedom	Wilk's lambda Λ	F approximation
Treatment	H	$h = (t-1)$	$\frac{ R }{ H+R }$	Equation 1
Residual	R	$r = t(n-1)$	-	-
TOTAL	T Sum of above	$t = tn-1$	-	-

There are no quantities which correspond to the mean squares (M) of ANOVA, instead the test statistic, termed Wilks' Λ -Lambda (Λ) is calculated as the ratio of the determinant of matrix R divided by the determinant of the matrix which is the sum of both H and R . The F -statistic approximation and its degrees of freedom are then calculated as described in equation 1.

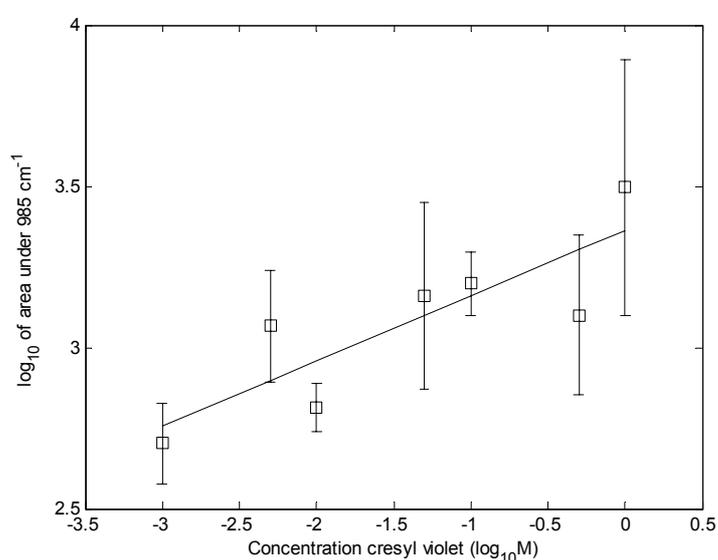
$$\frac{1 - \Lambda^{1/b}}{\Lambda^{1/b}} \cdot \frac{(ab - c)}{ph} \approx F_{[ph, ab-c]} \quad (\text{Eq. 1})$$

$$\text{Where } a = \left(r - \frac{p-h+1}{2} \right), \quad b = \sqrt{\frac{p^2 h^2 - 4}{p^2 + h^2 - 5}}, \quad c = \frac{ph-2}{2}$$

and h , r and t are defined in Table 1b and p is the number of variates.

Quantification of Cresyl Violet by SERRS

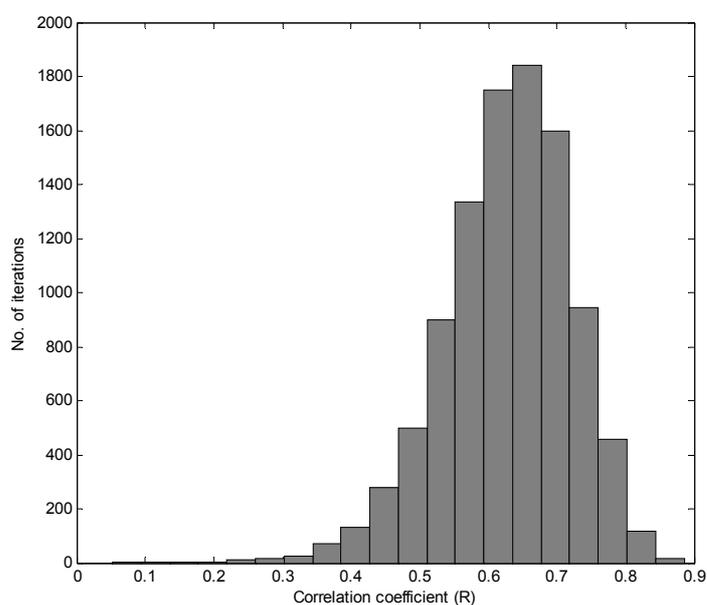
Due to the reproducibility of the raw SERRS spectra, PVP capped colloidal silver was assessed for quantitative SERRS analysis of cresyl violet. Using a single batch of this sol, SERRS spectra of cresyl violet were acquired over seven concentrations, ranging from 5×10^{-6} M to 5×10^{-4} M.



Supplementary Figure 2. The log-log calibration model for concentrations of cresyl violet ranging from 5×10^{-6} M to 5×10^{-2} M. The area under the band at 985 cm^{-1} was used and the mean with standard deviation error bars for each point on the calibration curve are plotted with the least squares fit straight line ($y = 0.203x + 3.364$).

Supplementary Table 3. R values and linear coefficients for calibration models calculated from all bands in the SERRS spectrum of cresyl violet.

Raman shift (cm ⁻¹)	Correlation coefficient (R)	Linear equation
846	0.841	$y = 0.195x + 3.324$
875	0.841	$y = 0.195x + 3.332$
985	0.847	$y = 0.203x + 3.364$
1049	0.845	$y = 0.203x + 3.280$
1105	0.845	$y = 0.209x + 3.344$
1228	0.807	$y = 0.176x + 3.397$
1277	0.784	$y = 0.170x + 3.303$



Supplementary Figure 3. A histogram representing the results of a bootstrap analysis based on calculation of the correlation coefficient for the log-log relationship to area under the cresyl violet SERRS band at 930 cm⁻¹ for dilutions ranging from 5 x 10⁻⁶ M to 5 x 10⁻² M, using the PVP capped colloidal silver substrate.

In Supplementary Figure 2, the regression line shows the log-log relationship between the means and standard deviations of S/N for the band at 985 cm⁻¹ and cresyl violet concentration (R=0.84). Clearly, a correlation between S/N for this band and analyte concentration exists, but is subject to error. This relationship is similarly observed when taking any of the SERRS peaks in to account, as can be seen from Supplementary Table 3. To further investigate the robustness of this model with respect to the peak at 985 cm⁻¹, a bootstrap analysis (Supplementary Figure 3) was performed¹¹. This involves iteratively modelling (in this case 10³ iterations) on randomly selected subsets of the logged S/N measurements with replacement, to build up a picture of the accuracy of the model based on the full set of data. The correlation coefficient is calculated for each of these models and the distribution of R values plotted as a histogram. Encouragingly, from Supplementary Figure 3 it can be seen that the position of the model is centred in the region of R = 0.7 - 0.8, which demonstrates good model stability.

References

- 1 P.C. Lee; D. Meisel *J. Phys. Chem.* 1982, **86**, 3391.
- 2 V. Fabrikanos; S. Athanassiou; K.Z. Leiser *Naturforsch. B* 1963, **18**, 612.
- 3 S. Panigrahi; S. Kundu; S. Ghosh; S. Nath; T. Pal *J Nanopart Res* 2004, **6**, 411.
- 4 H. Hiramatsu; F. Osterloh *Chem. Mater.* 2004, **16**, 2509.
- 5 Y. Sun; Y. Xia *Science* 2002, **298**, 2176.
- 6 B. Korgel; D. Fitzmaurice *Phys Rev B* 1999, **59**, 14191.
- 7 C. H. Munro; W. E. Smith; M. Garner; J. Clarkson; P.C. White *Langmuir* 1995, **11**, 3712.
- 8 R. Fisher *Ann. Eugen.* 1936, **7**, 179.
- 9 L. Stahle; S. Wold *Chemomet. Intell. Lab. Sys.* 1990, **9**, 127.
- 10 H. Johnson; A. Lloyd; L. Mur; A. Smith; D. Causton *Metabolomics* 2007, **3**, 517.
- 11 B. Efron; R. Tibshirani *Statist. Science* 1986, **1**, 54.