

Differentiating Intrinsic SERS Spectra from a Mixture by Sampling Induced Composition Gradient and Independent Component Analysis

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Supplementary Material

Various mixtures of BPE and MPh were prepared and 4 μl of each mixture was applied to a separate substrate. After the solvent had dried multiple points ($n = 15$) were measured on each substrate using the same measurement conditions previously outlined in this report. The average spectra for each mixture-treated substrate is determined and used to ascertain the individual BPE and MPh component spectra y_1 and y_2 , respectively. Several different reference samples were used, BPE:MPh = 100:0, 50:50, or 0:100, as x_1 . Each reference was then compared to each of the 10 other mixtures (x_2) to generate y_1 and y_2 . The estimated y_1 and y_2 spectra were then compared to the s_1 and s_2 (i.e. 100:0 and 0:100 BPE:MPh, respectively) and representative results are demonstrated in Fig. S2A and S2B for BPE and MPh, respectively.

For most of the estimated spectra we can see a very high degree of similarity between the estimated y_1 spectra and the pure analyte signal. The high degree of cross correlation r obtained for y_1 and y_2 with s_1 and s_2 , respectively, quantitatively demonstrate very robust and accurate separation of the component signals. A plot of the r values as a function of BPE:MPh using three different reference samples is shown in Fig. S3. These plots show that the pure BPE and pure MPh are the best references to use to obtain the most accurate BPE and MPh component signals, respectively. Incidentally, using these pure samples as references yields the worst component spectra when determining the source signal of the opposite analyte when it is present at lower concentrations. Using the 50:50 BPE:MPh sample as a reference appears to work sufficiently well for both analytes at all concentrations.

Because the weighting coefficient a_{ij} represents a (relative) quantitative measure of the component signal, we compared this value to the measured intensity of the source signal x_i . Figure S4A shows the normalized ratios of the calculated mixing coefficient. For each mixture, the BPE weighting coefficient a_{21} was calculated and then divided by the reference weighting coefficient a_{11} . Just as with the spatial mapping data in Fig. 3, all a_{21}/a_{11} ratios for a given reference were divided by the maximum

a_{21}/a_{11} value of that reference set to make the data more comparable. These normalized values are then compared to the measured I_{BPE} . Because ICA does not generate weighting coefficients when the measurement is the same as the reference measurement (i.e. when $x_1 = x_2$) we point out that we artificially set $a_{21}/a_{11} = 1$ for the reference sample, just as with the ratios in Fig. 3. We also note that occasionally FastICA generates negative weighting coefficients, which is likely resulting from noise; however, simply taking the absolute value of the estimated a_{ij} appears to mitigate the issue.

Based on Fig. S4A, ICA clearly demonstrates an accurate and precise method for quantitatively determining the relative contribution of the BPE component signal from a mixture. Furthermore, regardless of which reference sample is used ($x_1 = 100:0$, $0:100$, or $50:50$ BPE:MPH), the results correspond very closely with the measured intensity. Figure S4B shows the same treatment for MPH (i.e., a_{22}/a_{12}) as in part (A). We point out that the MPH weighting coefficient ratio appears to deviate slightly more from the measured I_{MPH} compared to BPE, but still follows the same overall trend of the I_{MPH} . This is similar to Fig. 3B which also demonstrates some deviation of a_{22}/a_{12} from the measured intensity, but the exact cause for this discrepancy between the accuracy of the BPE and MPH is still unclear.

Using the estimated weighting coefficients used in Fig. S4 we can construct a calibration curve to relate the ratio of the BPE and MPH weighting coefficients a_{21} and a_{22} , respectively, to their molar ratio for a given measurement location from Fig. 3. To do this, the a_{21}/a_{22} ratio (using values estimated with 50:50 BPE:MPH reference) were plotted versus the molar ratio. We note that only the 9 intermediate mixture samples were used, and pure samples (100:0 and 0:100) were not included in the calibration. The result is shown in Fig. S5A, and we can see that the resulting plot is roughly linear. Due to the fact that a_{21}/a_{22} is not perfectly monotonic with respect to the BPE:MPH ratio the calibration plot shows some inconsistencies (i.e. doubling back on itself) when a_{21}/a_{22} becomes small. Regardless, a suitable linear fit is achievable which is displayed with its equation in Fig. S5A. To relate the ratio of the weighting coefficient to the molar ratio, the a_{21}/a_{22} values from Fig. 3A and 3B were input into this equation, yielding a BPE:MPH molar ratio for each location, which is plotted in Fig. 4. For comparison, another calibration plot of the measured intensity ratio $I_{\text{BPE}}/I_{\text{MPH}}$ vs. BPE:MPH molar ratio is shown in Fig. S5B. This plot is very similar to the calibration curve determined for a_{21}/a_{22} ; likewise, the linear fit is also similar.

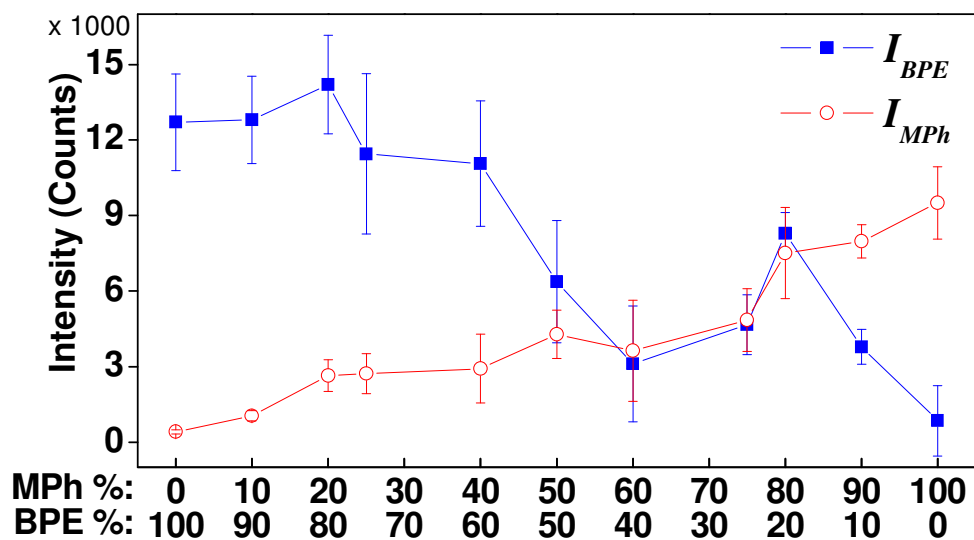


Fig. S1. Measured intensity of BPE and MPh as a function of their respective concentration ratios. Each data point is an average of multiple points ($n = 15$) on a single substrate; the error bars represent one standard deviation.

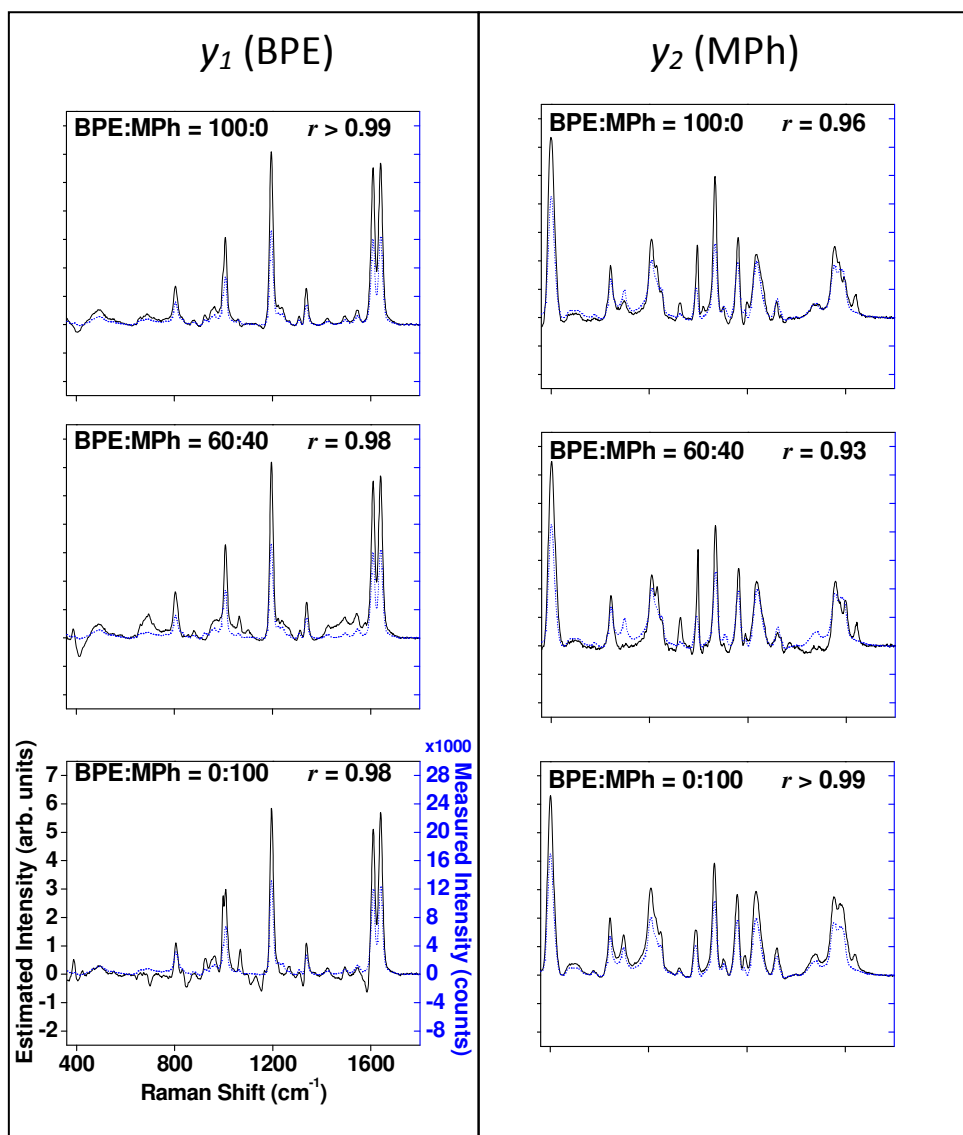


Fig. S2. Comparison of representative estimated spectra (solid line) of y_1 (left) and y_2 (right) compared to the pure source signal (dotted line) for different mixture ratios. Spectra were generated using the references $x_1 = 50:50$ BPE:MPh. The correlation coefficient r is presented for each plot. Scale is identical for all plots.

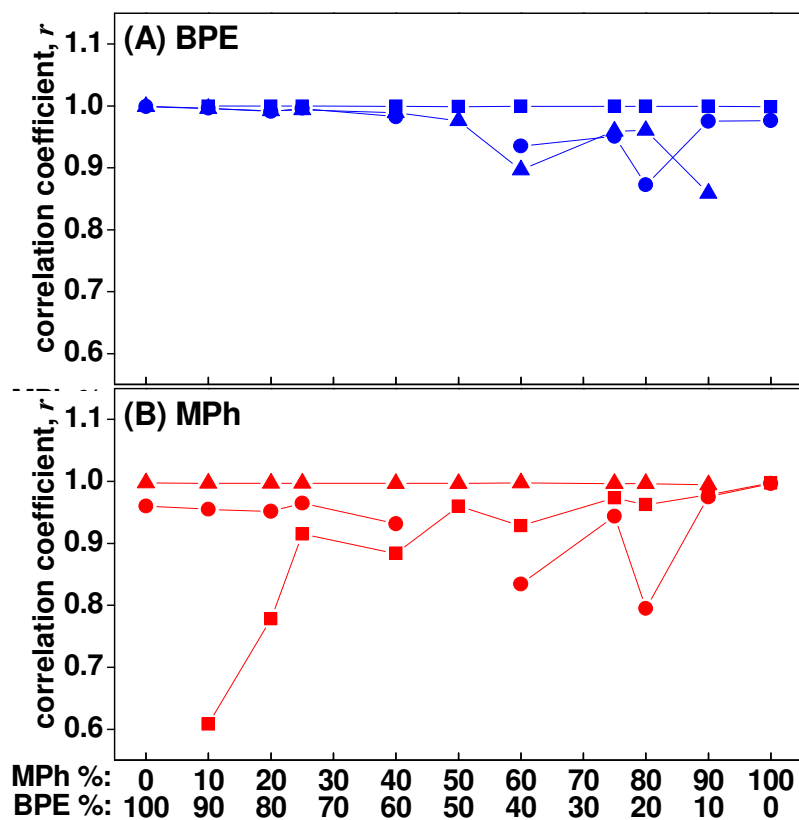


Fig. S3. Correlation coefficients r vs BPE:MPh mixture samples for A) y_1 vs. 100:0 BPE:MPh and B) y_2 vs. 0:100 BPE:MPh. Correlation coefficients were generated for y_1 and y_2 vs pure BPE and MPh samples using different x_1 reference samples: (!) – 100:0, (,) – 50:50, and (Ω) – 0:100 BPE:MPh.

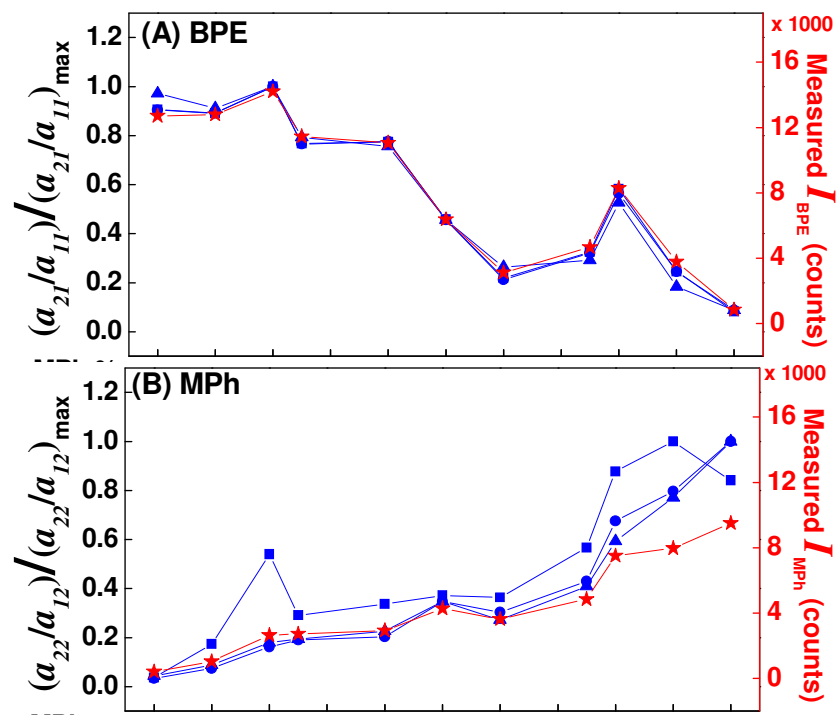


Fig. S4. A) Comparison of the normalized a_{21}/a_{11} ratio and the measured I_{BPE} as a function of BPE:MPh mixture ratio. The normalized a_{21} and a_{11} are obtained using different x_1 reference samples: (!) – 100:0, (,) – 50:50, and (Ω) – 0:100 BPE:MPh; I_{BPE} is represented with the (ξ). B) Same as (A) but using normalized a_{22}/a_{12} and I_{MPh} .

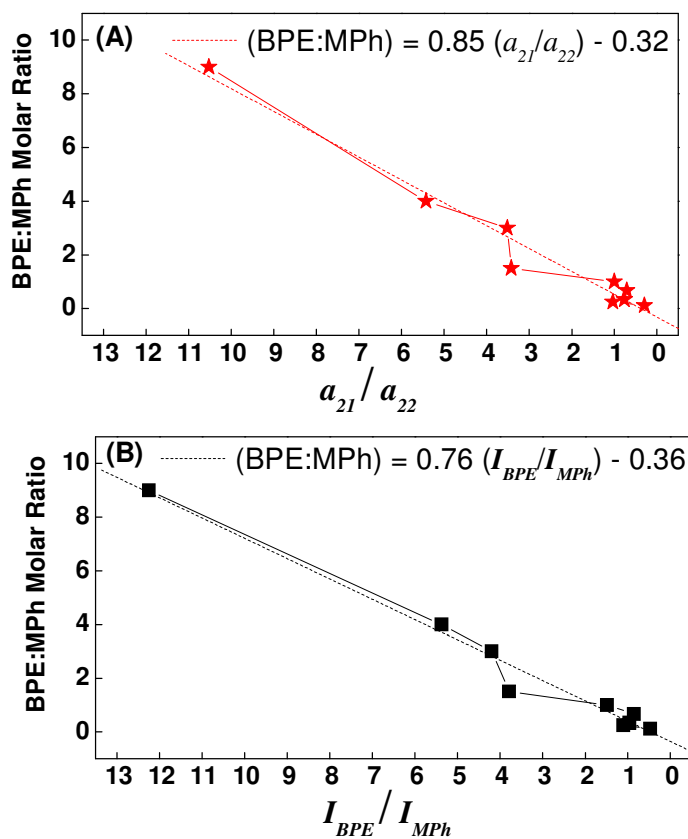


Fig. S5. Calibration plot for A) a_{21}/a_{22} and B) $I_{\text{BPE}}/I_{\text{MPh}}$ versus BPE:MPH molar ratio. The weighting coefficients a_{21} and a_{22} were obtained from the 50:50 BPE:MPH reference data. The dotted lines represent linear fits of the data and their respective equations are displayed. Note that the pure BPE and MPh samples were not used to determine the calibration curve.