A127 Comparison of Aggregating Agents for Surface-Enhanced Raman Analysis of Benzodiazepines

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The goal of this presentation is to show attendees the applicability of surface enhanced Raman spectroscopy to the analysis and detection of trace quantities of benzodiazepines. The optimization of various parameters of this technique as well as the limits of this method will also be discussed.

This presentation will impact the forensic science community by showing a technique that has better selectivity and sensitivity than current immunoassay screening techniques for benzodiazepines.

Benzodiazepines are commonly prescribed medications for anti-anxiety and anti-depression. The effects these compounds have on the central nervous system such as drowsiness, amnesia, confusion, and impaired coordination have made these drugs prominent in the commission of drug-facilitated sexual assaults. There has been a significant increase in the prevalence of these types of drugs in case submissions. The target detection limit for these compounds in biological samples is 50ng/mL, which is well below therapeutic concentrations.

Surface-Enhanced Raman spectroscopy (SERS) has previously been shown to detect trace quantities of compounds, such as nicotine, in aqueous solutions. This technique has the advantage of overcoming the low sensitivity and quenching the unwanted fluorescence effects seen with conventional Raman spectroscopy. SERS spectra are obtained by applying a compound of interest onto a SERS-active metal substrate such as colloidal metal particles or metal films. In this case, the colloidal particles are spherical gold nanoparticles in aqueous solution.

To further increase the enhancement of the SERS signal, aggregate solutions are used. These agents are salt solutions which cause the nanoparticles to amass and form hot-spots which increase the signal intensity. Chlorine salts generally provide the greatest enhancement for two reasons. The chlorine ions displace the stabilizing agent to cause aggregation and they affect the ionic strength of the surrounding solution changing the surface charge of the substrate, therefore increasing the signal intensity. While a single aggregating salt will affect a substrate the same, it has different effect on the signal of various analytes. Aggregating agents must be assessed for each individual drug to determine the optimum aggregating agent for a range of benzodiazepines.

Aqueous colloidal dispersions of gold spherical nanoparticles were prepared using a modified Lee Meisel 1% sodium citrate reduction method. Particle size and shape were confirmed with an average size of approximately 30 nm. Diluted benzodiazepine and metabolite samples were prepared in 10% methanol. Four aggregating agents were compared for enhancement of spectral characteristics. $MgCl_2$, $CaCl_2$, NaCl, and KCl were chosen and prepared at a concentration of 1.67 M. Aggregate solutions were added to colloidal dispersions followed by the addition of a range of benzodiazepine concentrations (1ng/mL – 1000ng/mL) and SERS spectra were obtained.

It was found that each aggregate had different enhancement effects on each individual drug. Overall $MgCl_2$ provided the lowest limit of detection, 2.5ng/mL, and linearity over a range of concentrations for a variety of drugs chosen. It was also observed that generally the higher the chlorine ion concentration, the higher the SERS intensity observed. Lastly, the cations of the aggregating solutions had an effect on the SERS signal. The smaller the cation the higher the intensity produced.

This method has shown the applicability of SERS for the detection of trace quantities of benzodiazepines in aqueous solutions as well as the optimization of the technique over a wide range of compounds. This technique can be adapted for use in the detection of trace benzodiazepines in toxicological samples such as urine. SERS is more specific than currently used immunoassays giving spectral information about the compound present. Also, this technique has more sensitivity than immunoassays and in the case of benzodiazepines such as lorazepam that have poor cross-reactivity, the drug can be detected.

Benzodiazepine, Surface-Enhanced Raman Spectroscopy,
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