EX-SITU REAL-TIME DETECTION OF NEUROTRANSMITTERS IN PERFUSION DIALYSATE

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Abstract: Spatial distributions of neurotransmitter levels determine ones psychological and emotional well being. The determination of these levels is difficult due to the complex cerebral spinal fluid matrix and the low trace levels of neurotransmitters present.
We will present results from a spectroscopic assay for neurotransmitters that can be performed in real-time as neurotransmitters are dialysized from a rat brain.

Spatial distributions of neurotransmitter levels determine one's psychological and emotional well being. The determination of these levels is difficult due to the complex cerebral spinal fluid matrix and the low trace levels of neurotransmitters present. In practice, psychiatric researchers not only want to know the level of a neurotransmitter, but also its temporal changes due to drug therapy and the levels of several neurotransmitters and their metabolites simultaneously. We will present results from a spectroscopic assay for neurotransmitters that can be performed in real-time as neurotransmitters are dialysized from a rat brain. The technique relies on SERS detection of an azo adduct of the neurotransmitter. Azo adducts are formed by reaction with a reactive diazonium probe that contains an argentiphilic group. We will show chemometric results that both separate and quantitate different neurotransmitters and their metabolites simultaneously.

Neurotransmitters (NT) levels can be monitored using SERS and the application of reactive coatings specifically designed to react with the NT and silver surfaces giving the selectivity and sensitivity needed for real-time *ex-vivo* monitoring. The normal Raman spectrum of each of the NTs demonstrates that there are unique spectral features for each species. This enables the simultaneous detection of multiple NTs and/or their metabolites.

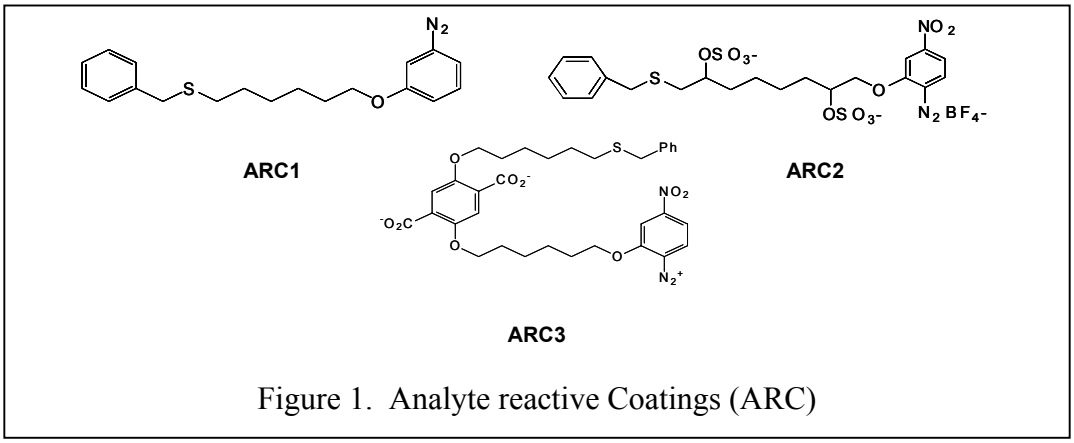

Figure 1. Analyte reactive Coatings (ARC)

Figure 1 shows three Analyte Reactive Coatings (ARCs) developed that have two reactive sites; one diazonium for the NT, and the other for attachment to the silver. We have used benzylmercaptan as the argentiphillic group. It has been shown that silver surfaces specifically cleave the bond between the benzylic carbon and the sulfur.(1) The reaction between the NT and the diazonium creates an azo dye much like that of many common dyes. The creation of the dye

makes the compound resonance Raman active that in turn leads to higher sensitivity. The reaction between the NT, the ARC and the silver surface allows for an enhanced signal and lower detection limits of NTs and their metabolites. Additionally, with the structural sensitivity of Raman spectroscopy, it is possible to monitor multiple NTs or an NT and it's metabolite simultaneously. This is accomplished by assigning unique spectral areas to each of the analytes. Substitution of constituents on the diazonium allows the λ_{max} to be tuned to the desired wavelength (632.8nm) that in turn increases the sensitivity up to a thousandfold. With the 10^7 enhancement of SERS and the added enhancement of 10^3 the over all enhancement may be as high as 10^{10}.(2-4)

Preliminary with serotonin is shown in Figure 2. A flow injection system was created to test the feasibility of an *ex-vivo* process involving perfusion of NTs. This cell provided time resolved monitoring of NTs and their metabolites in perfusion dialysate. A resolution time of 10 seconds was achieved for this system with serotonin levels around 100 nM. The nanomolar levels of serotonin can be seen in Figure 2.

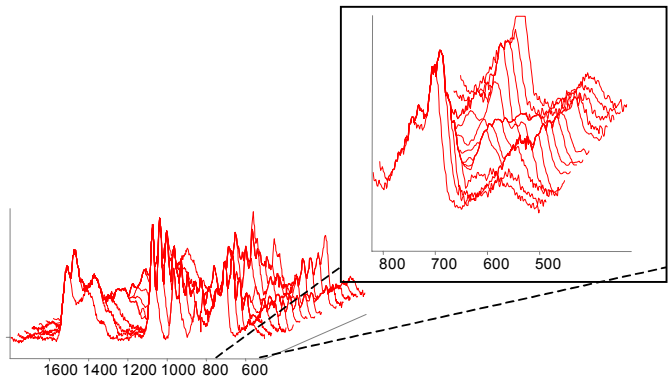

Figure 2. 5µL injection of 100nm Serotonin reacted with ARC2.

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References:

1. CJ Sandroff, DR Herschbach: Surface-enhanced Raman study of organic sulfides adsorbed on silver: Facile cleavage of sulfur-sulfur and carbon-sulfur bonds. Journal of Physical Chemistry 86 (1982) 3391-5.
2. M Moskovits: Surface-enhanced spectroscopy. Reviews of Modern Physics 57 (1985) 783-826.
3. K Kneipp, H Kneipp, I Itzkan, RR Dasari, MS Feld: Ultrasensitive chemical analysis by Raman spectroscopy. Chemical Reviews (Washington, D. C.) 99 (1999) 2957-75.
4. EJ Zeman, KT Carron, GC Schatz, RP Van Dyne: A surfaced enhanced resonance Raman study of cobalt phthalocyanine on rough Ag films: Theory and experiment. Journal of chemical Physics 87 (1987) 4189-200.