Surface Enhanced Raman Spectroscopy of Neurotransmitters

Michael L. McGlashen, Kevin L. Davis and Michael D. Morris*
The University of Michigan Chemistry Department
Ann Arbor, Michigan 48105

Abstract

The surface-enhanced Raman spectra (SERS) of neurotransmitters in biological matrices and synthetic solutions are described. The effects of protein adsorption on catecholamine SERS intensity are discussed. Techniques for obtaining dopamine SERS spectra in cerebrospinal fluid and rat brain dialysate are demonstrated. Preliminary SERS of histamine and tele-methylhistamine are presented.

Introduction

There are about two hundred molecules which are known or suspected to function as neurotransmitters.[1-3] These include several aromatic amines, such as the catecholamines, histamine and serotonin, several amino acids, and a large number of oligo- and polypeptides. In addition, neurobiologists and pharmacologists have studied numerous closely related molecules, either as pharmaceuticals, or as probes of the important features of neurotransmitter and receptor function and structure.

Because the synaptic cleft in mammalian neurons is no more than 20 nm across, neither optical or physical probes of neurotransmitter concentrations in this region are practical. Instead, researchers have usually relied on probes placed in the extracellular fluid.

Three general methods of real-time neurotransmitter measurement are flow perfusion, dialysis perfusion and voltammetry.[4] Flow perfusion techniques are based on the diffusion of materials into a stream of synthetic cerebrospinal fluid flowing into and out of the test area. The stream is pumped from a reservoir into a small tube (cannula) inserted into the test area of the brain or brain slice and removed by pumping from a concentric cannula placed outside the input tube. The time constants are reasonably fast (10 sec), but materials are badly diluted by the flow process. Any convenient assay technique can be used on the perfusate. Immunoassay, liquid chromatography with electrochemical or fluorescence detection and gas chromatography/mass spectrometry are among the common finishes.

Dialysis perfusion techniques are based on transport of the neurotransmitter across a membrane which terminates a perfusion probe. The neurotransmitters are isolated and measured externally. Membranes with a molecular weight cutoff of 5,000 a.m.u. and lower have been used.[5,6] Dialysis perfusion is attractive because the protein burden of the sample is greatly reduced. In addition, dialysis decreases the extent of tissue damage at the probe site because the tissue is not directly exposed to fluid flow. The assay techniques are the same as those used in push-pull perfusion.

Measurements of catecholamines, serotonin and their metabolites can be made by anodic voltammetry at a carbon electrode.[4,7] Voltammetry provides close to real-time response. Carbon electrodes range in size from 40–50 μm diameter graphite epoxy disks[7] to 1 μm diameter carbon fibers[8]. Response times are a few seconds.[9]

Voltammetry is not problem free. It can be used only with catecholamines and serotonin, and some of their metabolites, precursors or synthetic analogs. Further, the range of oxidation potentials within a class of molecules is small. In general, a neurotransmitter and its metabolites or precursors can not be completely resolved voltammetrically[7].

In addition, ascorbic acid is more easily oxidized than catecholamines or serotonin. On untreated carbon electrodes, an ascorbate signal badly overlaps catecholamine signals. The problem can be overcome in several ways. Two effective methods are the use of chemically treated carbon paste electrodes[10] and use of Nafion coated graphite electrodes.[11]

Histamine presents a special analytical challenge. It is not electroactive and it absorbs deep in the UV, $\lambda_{max} = 207$ nm, making LC detection difficult without derivatization. Radioenzymatic assay remains the commonly used finish to histamine perfusion studies. Histamine assays are notoriously difficult. Problems arise in part from the assay itself and in part from the variability in histamine content with the method used to sacrifice the test animal.[12]

Theory

Surface enhancement of Raman spectra occurs as the result of several processes which take place on structured metal surfaces[13]. These can be divided into two classes: electromagnetic effects[14,15] and chemical (or charge transfer) effects.[16] Since chemical enhancement is normally accompanied by electromagnetic enhancement, the total enhancement is the product of both processes. In this case, good spectra can be observed from molecules adsorbed from $10^{-6}M$ solutions. Detection limits are for adsorption from solutions 10^{-7} to $10^{-8}M$.

Biogenic amine neurotransmitters such as dopamine, histamine and serotonin are nearly ideal SERS substrates. As aromatic amines, they form strong complexes with silver, copper or gold. Charge transfer enhancement contributes to good SERS sensitivity. Each neurotransmitter can be viewed as an aromatic ring system with different substituents defining the neurotransmitter and its metabolites. Distinguishing among such compounds is a classic strength of vibrational spectroscopy.

Hsieh *et al.*[17] have recently reported the SERS spectra of the naturally–occuring catecholamines and some metabolites and analogs on silver electrodes. They demonstrated that the major bands in the spectra were aromatic ring modes due to the catechol moiety. Table I summarizes band positions and assignments in that system.

Table I.
SERS shifts (cm^{-1}) of catecholamines and related compounds.

bands	DA[a]	NOR[a]	MTA[a]	catechol
ν_{15}	1152	1150		1149
ν_{C-O}	1269	1272	1273	1258
ν_3	1331	1329	1363	1331
ν_{19a}	1424	1422	1441	
ν_{19b}	1479	1479		1469
ν_{8a}	1572		1578	
ν_{8b}	1584		1601	

[a]Abbreviations: DA, dopamine; NOR, norepinephrine; MTA 3-methoxytyramine.

The intense band near 1480cm^{-1} has been assigned[17] to the benzene ν_{19b} mode. This band is generally the most convenient for quantitative measurements. Its absence distinguishes the methoxylated compounds from the catechol compounds. The band near 1270 cm^{-1} is the phenolic C–O stretch. It may also be used for quantification.

Although the band positions in dopamine and norepinephrine are quite similar, the relative intensities are not. For example, ν_3 is strong in dopamine spectra, but quite weak in norepinephrine spectra. Thus, band intensity ratios can be used to distinguish dopamine from norepinephrine.

Catecholamines with highly substituted ethylamine side chains give weak spectra. Bulky substituents apparently force an unfavorable adsorbate orientation. Spectra of anionic (acetate) metabolites have not been observed. For strong catecholamine adsorption, the electrode potential must be near the potential of zero charge. Under these conditions, anions are desorbed from the electrode surface.

Hsieh *et al.*[17] worked only in synthetic electrolyte solutions. In the brain, however, the extracellular fluid (ECF) contains electrolytes as well as small amounts of serum albumin, immunoglobins and other proteins. Cerebrospinal fluid is essentially the equivalent of the brain ECF. Other neurochemical samples, such as extracts of tissue homogenates, contain high protein burdens as well as lipids and other surface-active molecules. Because these surfactants could interfere with SERS measurements, it is important to investigate neurotransmitter SERS in these media.

Catecholamines in Neurochemical Media

We present catecholamine SERS obtained in several neurochemical media using silver electrodes. The experimental apparatus and procedures were generally similar to those described by Hsieh *et al.*[17] The silver electrode was maintained at −0.9 V vs. S.C.E. For all experiments, a Spex Triplemate spectrograph equipped with a Tracor Northern or a PARC 1420 intensified diode array detector was employed. Integration times of 100 sec were used. Spectra were obtained using 30 mW of Ar^+ 514.5 nm excitation. Sample volumes were 0.2–0.5 ml.

We have investigated the effects of protein adsorption on catecholamine SERS intensity. For this work, we have used albumin, which is the major protein in cerebrospinal fluid. Figure 1 shows the effect of albumin addition on dopamine SERS intensity. At 0.5% albumin concentration, SERS intensity is reduced to roughly half its value in protein-free solutions, and there is a clear decrease in the quality of the spectrum. When the protein burden reaches 1%, the spectrum is barely visible.

Figure 2 shows the SERS spectrum of cerebrospinal fluid (CSF, Sigma Diagnostics) spiked with approximately $2 \times 10^{-5} M$ dopamine. This concentration is near the upper end of the useful range for neurochemistry. The four major bands of the dopamine SERS spectrum are present but the signal magnitude and signal/noise ratio are slightly less than in the simple electrolyte solutions used by Hsieh *et.al.*[17]

In general, CSF has a protein concentration of between 15 and 40 mg per 100ml.[18] The assay value for the sample used here is 31 mg/100ml (0.31%). About 65% of the protein is serum albumin. CSF is viscous and the protein surfactants can cause extensive foaming. Consequently, CSF requires more careful handling than synthetic electrolyte solutions which contain only small molecules or ions. Standard addition may be used for quantification. However, the addition must be followed by thorough stirring to avoid artifacts. The Sigma CSF samples also contain 0.05% sodium azide as a preservative. The effects of azide, if any, are not yet known.

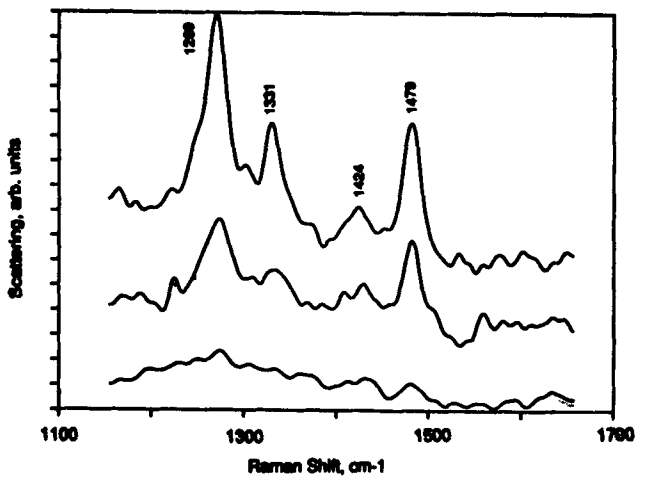

Figure 1. Effects of Albumin Concentration on Catecholamine SERS Intensity at pH 7.
Top: 0%, Middle: 0.5%, Bottom: 1.0%

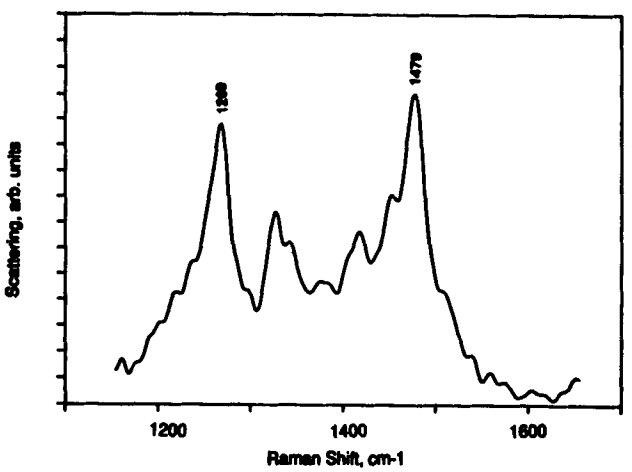

Figure 2. SERS Spectrum of Cerebrospinal Fluid, Spiked with $2 \times 10^{-5} M$ Dopamine.

Figure 3 shows the spectrum of a dialysate sample obtained from a rat brain striatum by Professors Jill Becker and Terry Robinson (University of Michigan, Department of Psychology). The sample is spiked with $5 \times 10^{-6} M$ dopamine. With its low residual protein content, the dialysate sample is nearly the equivalent of a synthetic electrolyte matrix. The signal magnitude and signal/noise ratio approach those obtained with simple aqueous solutions.

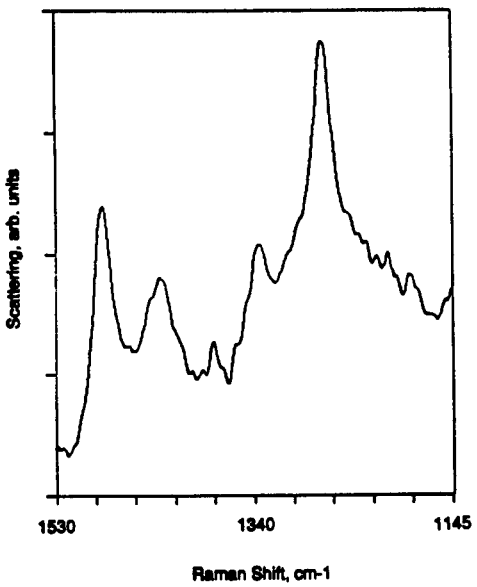

Figure 3. SERS of Rat Brain Striatum Dialysate, Spiked with $5 \times 10^{-6} M$ Dopamine.

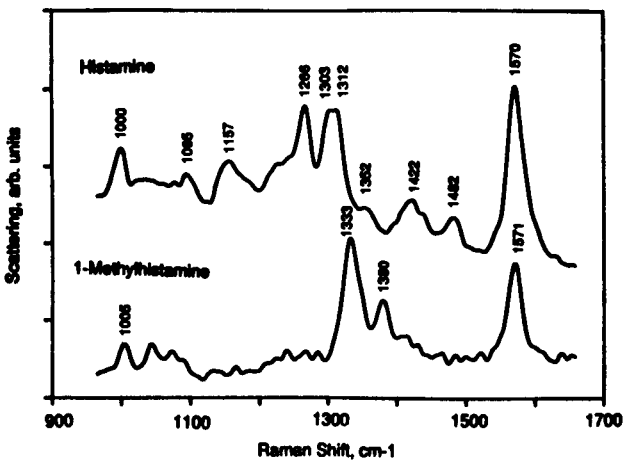

Figure 4. SERS Spectra of Histamine and *tele*-Methlyhistamine at pH 7.

Histamine

We have also made preliminary SERS measurements of histamine and its primary metabolite tele-methyl histamine on a silver electrode. As shown in Figure 4, the spectra of histamine and its metabolite are readily distinguished. In particular, the intense histamine bands at 1266, 1302 and 1312 cm^{-1} are missing in the methylated compound, which has the single strong 1332 cm^{-1} band in this region. The signal magnitudes and signal/noise ratios obtained in these spectra are similar to those of the catecholamines.

Conclusions

We are actively developing SERS as a multi-purpose probe of neurotransmitter concentrations. We have verified the feasibility of catecholamine SERS analysis of real biological matrices and have demonstrated useful SERS from histamine and its methylated metabolite. We are developing new techniques to minimize protein interference. Very preliminary results with Nafion-coated electrodes suggest that excellent catecholamine SERS can be obtained from coated electrodes. This technique may prove the key to practical SERS measurements in a variety of neurochemical matrices.

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