Chemotherapy Drug Analysis in Saliva

RTA Application note # 01

Real-Time Analyzers' ***Simple SERS Syringe Capillaries*** consist of a metal-doped sol-gel immobilized in a glass capillary that can be optically accessed along its length to generate and collect surface-enhanced raman spectra (SERS). This allows performing rapid separations or extractions and SERS analyses of chemicals at concentrations as low as 1 part-per-billion (ppb) or 1 ng per ml. A simple two-step procedure is followed, in which a syringe is used to draw the sample solution through the sol gel and the Raman optical probe of our Industrial Raman Aanlyzer (IRA) scans the length of the sol-gel. The separation occurs in a few seconds, while spectra at multiple positions are collected in a few minutes.

Monitoring and controlling chemotherapy drug dosage

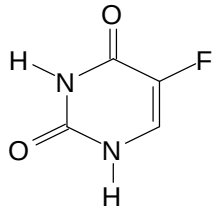
Determining safe and effective dosage for chemotherapy drugs remains a significant challenge in the treatment of cancer. Many of these drugs disrupt proper DNA and RNA replication leading to incomplete biochemical synthesis and cell death. The success of these drugs is based on the fact that cancer cells replicate more often than normal cells. However, some normal cells also turn over rapidly and adverse side-effects, such as myelosuppression in the case of blood cell production in bone marrow, can be life threatening. Furthermore, these dangerous side-effects preclude clinical trials, and the statistical basis for dosage is limited to the set of previously treated patients. A secondary approach to determining safe and effective dosage is to monitor its metabolism during administration and adjust concentrations accordingly. This information can be extremely beneficial since the patient's genetic makeup and nutritional habits can strongly influence the pharmacokinetics of these drugs. Unfortunately, current analyses require 10-20 cc of blood per measurement, and the multiple samples required to monitor drug metabolism may further jeopardize the patient's health, and, consequently, are rarely performed.

5-fluorouracil

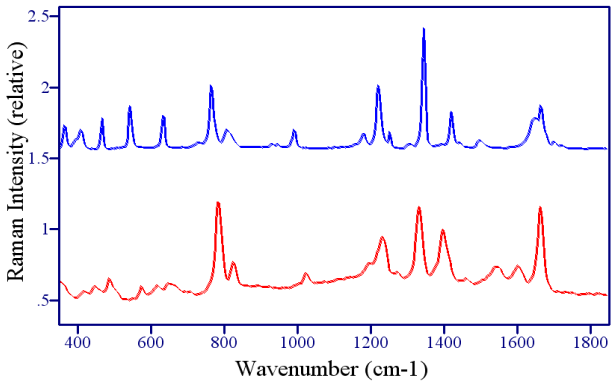
Saliva analysis has long been considered an attractive alternative, and recent research has shown that drug metabolism is often equally represented in saliva as it is in blood plasma, typically at mg/mL concentrations. Analysis of saliva is highly desirable in that it is easier to obtain and reduces the risk of HIV infection.

The drug compound 5-fluorouracil (5-FU) is one of the most often used drugs to treat solid tumors and colorectal carcinoma. However, there is a wide genetic-based variation in the metabolism of 5-FU, and administration of a "standard" dose of 5-FU has led to severe toxicity, even death.

We measured the surface-enhanced Raman spectrum of 5-FU and compared it to the normal Raman spectrum. Interaction with the silver surface causes some peaks to shift, such as 766 to 786 cm^{-1}, 1347 to 1335 cm^{-1} and 1669 to 1667 cm^{-1} for the pyrimidine ring-breathing mode, the N-H in-plane deformations, and the carbonyl stretches (a doublet in the Raman spectrum), respectively.



5-fluorouracil



Real-Time Analyzers, Inc.
362 Industrial Park Road Suite #8
Middletown, CT 06457
www.rta.biz

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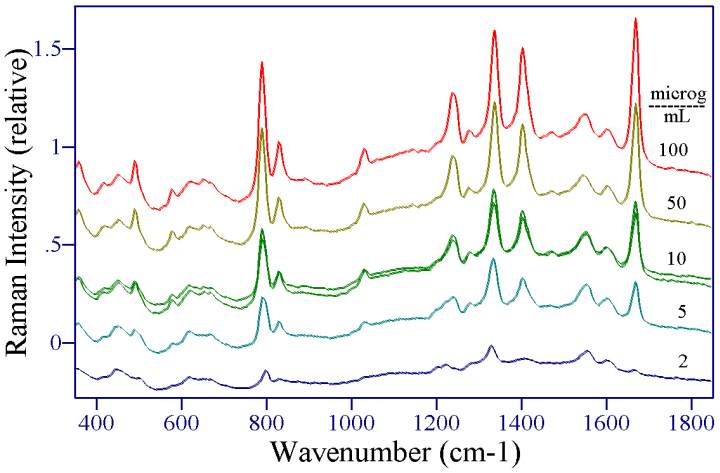
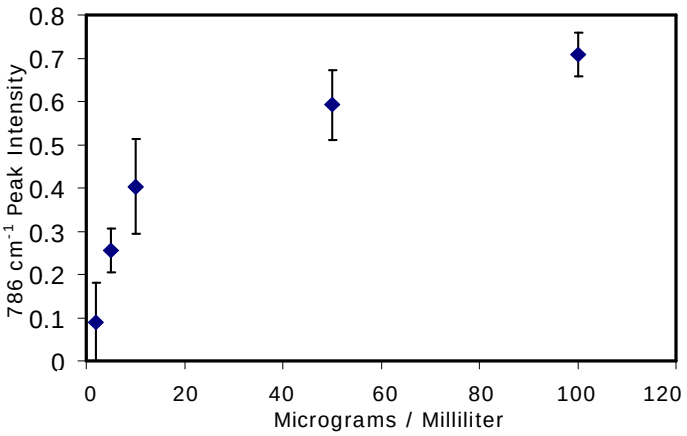
Physiological concentrations can be measured

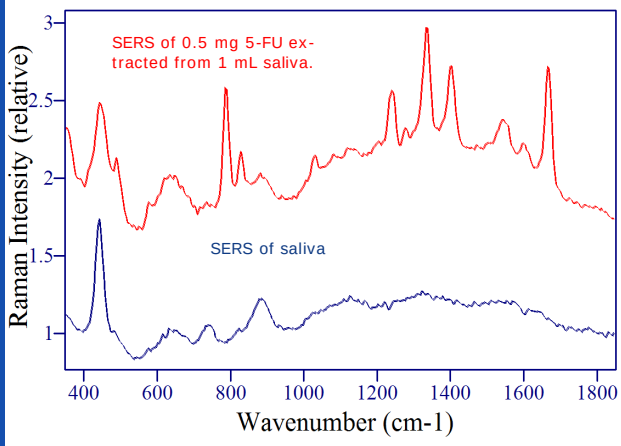
The SERS was quite intense, so samples were prepared and measured at lower and lower concentrations, down to 2 mg/mL. The height of the 786 cm^{-1} SERS peak was plotted as a function of concentration, which showed a Langmuir isotherm response. This is typical for SERS substrates where signal intensity is a function of available silver surface area. The quality of the SERS for the 2 mg/mL sample is quite good, and recently, we repeatedly measured 0.1 mg/mL in a 1 minute measurement. This sensitivity is within the 1-30 mg/mL expected range of physiological saliva concentrations.

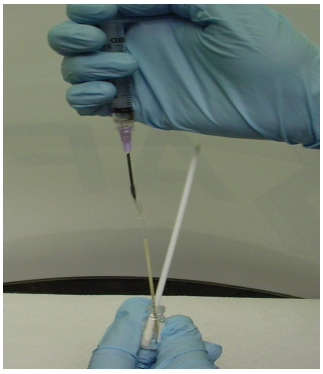


5-fluorouracil in saliva

A 1 mL saliva sample in which 0.5 mg of 5-FU was artificially added was drawn into a ***Simple SERS Syringe Capillary*** and placed on an ***Industrial Raman Analyzer*** and measured. All of the 5-FU spectral peaks were readily apparent. More recently we have measured 1 mg/mL of 5-FU in saliva. It is interesting to note that thiocyanate, sometime present in saliva, was also detected (peaks at 445 and 735 cm^{-1}). Nevertheless, the primary function of the sol-gels to separate 5-FU from the other constituents of saliva appears successful. For example, it is reasonable to assume that the large biochemicals of saliva, such as a-amylase and mucin (MW of approximately 50,000, and 10^6, respectively), do not diffuse through the sol-gel pores to the silver surface.







Drawing a 5-FU doped saliva sample into a ***Simple SERS Syringe Capillary.*** Less than a drop of saliva is needed.

See *Proc SPIE*, 5261, 135-141 (2004), *J Raman Spectrosc*, 36, 208-212 (2005) and *Vib Spectrosc*, 38, 79-84 (2005).

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