DEVELOPMENT OF A MICROFLUIDIC SYSTEM FOR THE SERS ANALYSIS OF ORGANICS ON ICY PLANETARY BODIES. M. Veneranda[1], A. Sanz-Arranz[1], C. Prieto Garcia[1], J.A. Manrique[1], O. Prieto Ballesteros[2], E. Charro[1], J.M. Lopez[1], M.A. Gonzalez[1], F. Rull[1], G. Lopez-Reyes[1]. [1] University of Valladolid (UVa), Valladolid Spain (marco.veneranda.87@gmail.com); [2] Astrobiology Centre (INTA-CSIC), Torrejon de Ardoz, Madrid, Spain

Introduction: NASA/Mars 2020 and ESA/ExoMars rover are equipped with the first Raman spectrometers to be ever used for space exploration missions (namely, SuperCam, Sherloc and RLS).

Looking ahead, further Raman prototypes are being developed for future rover missions to Europa (Jupiter's moon [1]) and other icy planetary bodies. The main scientific objectives of the future exploration missions are the characterization of (sub) superficial icy materials, and the identification of putative organic compounds possibly preserved therein.

In the past decades, several preliminary works have been carried out to assess the ability of Raman spectroscopy to discriminate the salt content of dissolved ice samples. However, in recent years, an increasing number of scientific articles have been published that propose analytical solutions that could increase the capability of Raman spectrometers to detect organics in icy planetary bodies.

In this framework, it is important to underline that Raman analysis performed in SERS mode (Surface-Enhanced Raman Spectroscopy) drastically reduce the Limit of Detection (LOD) of this technique, thus enabling the detection of organics in extremely low concentration values [2].

In the preliminary work published in this same issue (M.Veneranda et al.) a comparison between the LOD reached by different SERS nano emulsions and substrates has been carried out. Beyond confirming an extremely low LOD (from 10^{-6} to 10^{-11}, depending on the employed method), follow-up analysis of nucleotides solutions has shown the silver nanostructures suffer severe oxidation with time, thus leading to a drastic decrease of their plasmonic resonance effect after just a few weeks of aging. In order to overcome this issue and guarantee a constant plasmonic enhancement of silver nano emulsions throughout the whole time-frame of a space exploration mission (in the order of years), their synthesis should be performed directly on site right before running SERS analysis.

Working on this research line, the ERICA group has developed a novel microfluidic system allowing remote control of micropumps and microvalves, that can be used to synthesize silver nanostars emulsions and to perform SERS analysis of organic-bearing solutions directly on chip. In light of the potential application of this technology to the future exploration of icy planetary bodies, the present work seeks to 1) describe the characteristics of the microfluidic prototype, 2) compare the characteristics of the silver nanoemulsions synthesized by the microfluidic system with those obtained by conventional methods, and 3) test their plasmonic enhancement by determining the LOD of biomarker solutions.

Materials and methods: *Microfluidic components.* The microfluidic prototype was created by assembling commercial components. Microfluidic chips, connectors and tubing were purchased from Microfluidic ChipChop (Germany), while the piezoelectric micropumps and microvalves were purchased from Bartels Mikrotechnik (Germany).

Reagents: Silver nitrate ($AgNO_3$), sodium hydroxide (NaOH), hydroxylamine (HA) and citrate (CIT) were purchased from Sigma-Aldrich and diluted with MilliQ water to obtain the solutions necessary for the synthesis of silver nanostar emulsions [3]. Used as biomarkers, nucleotides (adenine, thymine, guanine, and cytosine) were also purchased from Sigma-Aldrich and dissolved in milliQ water to obtain solutions between $2\cdot10^{-1}$ and $2\cdot10^{-12}$ M.

Analytical instruments: The UV-Vis absorption spectra of synthesized silver nano emulsions was measured by the Lambda 35 system (Perkin Elmer), while the high-resolution scanning electron images of silver nanostructures were captured by the Quanta 200 ESEM FEG (FEI). After the synthesis of plasmonic nano emulsions, SERS analysis of dissolved biomarkers was carried out directly on chip by means of the InduRam (Horiba-JY) Raman spectrometer. The instrument is composed of a green excitation source (Laser Elforlight G4-30 PSU, emitting at 532 nm), while the spectrometer and the CCD detector are from a Horiba-JY Induram industrial Raman system.

Results: *Description of the microfluidic system.* The microfluidic system is provided with a passive mixer (staggered herringbone structure) to mix HA and NaOH solutions. The outlet of the passive mixer is connected to a mixing chamber equipped with a magnetic stirrer (active mixer). Here, the NaOH-HA solution is mixed with AgNO3 and CIT solutions which are connected to the active mixer through dedicated microfluidic channels. By following the procedure described in the dedicated patent (nº202131038), 250 µl of silver nanostars emulsion is obtained after about

20 minutes of mixing and stirring. An additional channel connects the active mixer to a second staggered herringbone structure, in which the silver nano emulsion is mixed with the organic-bearing solution to be investigated. After mixing, the resulting flow pass below a glass window, where the excitation laser of the Raman spectrometer is focused and the SERS signal is collected (see Figure 1).

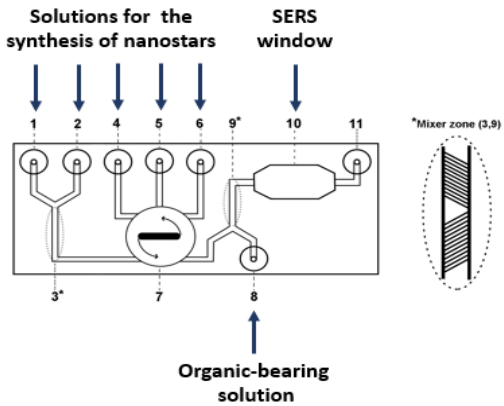

Figure 1: microfluidic prototype for the synthesis of silver nanostars and SERS analysis of organics.

The microfluidic prototype is coupled to piezoelectric micropumps and microvalves that are connected to reservoir tanks and automatically controlled by a dedicated software. In the current configuration, the microfluidic prototype is capable of synthesizing silver nanostars emulsions in a fully automated mode.

Analysis of microfluidic nano emulsions. As displayed in Figure 2, the UV-Vis spectra of the nanoemulsión synthesized by the microfluidic prototype fit quite well with those obtained by following the conventional laboratory procedure described by Garcia-Leis et al (2013). Indeed, both spectra present a main peak at 376 nm together with a shoulder on the right side.

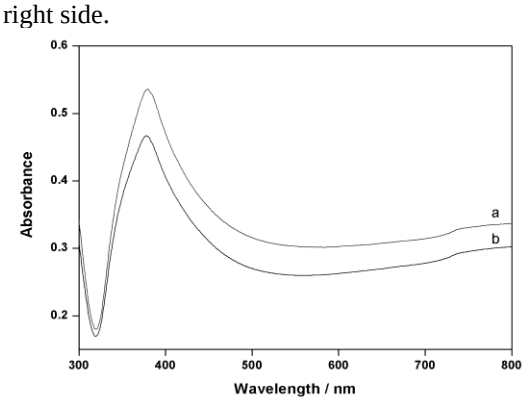

Figure 2: UV-Vis spectrum of microfluidic nanostars (a), compared to those obtained by following the conventional method of synthesis (b).

Complementary analysis were carried out by SEM to characterize and compare the synthesized nanostructures. As represented in Figure 3, the microfluidic nanostars have the same shape and size of the nanostructures presented in the literature [3].

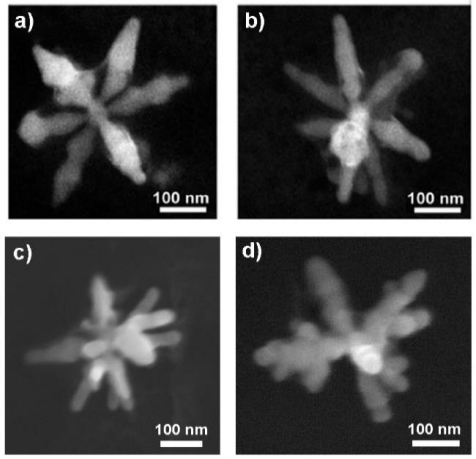

Figure 3: SEM images of silver nanostars synthesized by the conventional method (a and b) and by the microfluidic prototype (c and d).

SERS analysis of biomarkers on chip. After synthesizing the silver nano emulsion, the same microfluidic system was used to perform the SERS analysis of a set of water solutions bearing different concentration of adenine, thymine, guanine, and cytosine, respectively. Depending on the nucleotides under analysis, the characteristic peaks of the organic compounds were detected down to a molarity between 10^{-8} and 10^{-9}, thus achieving the same LOD obtained from silver nanostars emulsions synthesized by the conventional laboratory method (see the abstract of Veneranda et al. this issue).

Results: This work proves that, by developing tailored microfluidic devices, SERS analysis could be performed during planetary exploration missions. In light of the forthcoming rover exploration of Europa, this technology could be used to detect traces of organics potentially preserved at the (sub) surface of icy planetary bodies.

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References: [1] Sharma S. et al. (2020), *JRS, 9*, 1782-1793. [2] McNay D.G. et al. (2011), *App. Spectrosc., 65*, 825-837. [3] Garcia-Leis A. et al. (2013) *J. Phis. Chem. C., 117*, 7791-7795.