Problems of metrological support of Raman spectroscopy

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Abstract The development of Raman spectroscopy metrology is discussed. The modern efforts in this field are directed towards the support of quantitative analysis of substance concentration in volume and surface layers.

Raman spectroscopy is more and more widely used in the control of medicines, food products and food raw materials, in forensic medicine, forensic science and other areas subject to the government regulation in the field of ensuring the uniformity of measurements. This solves the problem of identifying substances by comparing the Raman spectrum measured for the reference sample with the spectrum (spectra) available in the database (library of Raman spectra). A significant problem that arises when comparing Raman spectra is related to the dependence of their shape on the characteristics of the Raman spectrometer or microscope on which they were measured, more precisely, on the spectral transmission of the collecting optics and the spectral sensitivity of the photodetector. Therefore, it is necessary either to use for comparison the spectra measured on the same instrument, which significantly limits the field of application of the method, or to calibrate the Raman spectrometers and microscopes on the intensity scale, while it is necessary to control the measurement accuracy on the spectral scale (frequency scale). To calibrate Raman spectrometers and microscopes in terms of intensities and to control their accuracy on a frequency scale, certified reference materials are required, which must be traceable to the primary state standard. The identification of substances by Raman spectra is a qualitative analysis. In quantitative analysis, to determine the concentration of substances by Raman spectra, it is necessary to determine the volume from which the scattered light is collected in a Raman microscope, the so-called "confocal volume"; for this purpose, reference materials are also required.

The leading metrological institutes of the world pay great attention to the metrological support of Raman scattering measurements, primarily the calibration of Raman spectrometers in terms of relative intensities. For these purposes, standard reference materials SRM 2242-2246 issued by NIST [1] are used. The use of these samples is governed by ASTM E2911. NIST-available Raman microscopes and/or Raman spectrometers are used to calibrate these reference materials and perform functions similar to those of the primary standard. Recommendations for the reproduction and transmission of frequencies (wave numbers) of Raman shifts are given in the ASTM E1840 standard. Further development of the metrology of Raman spectroscopy in leading metrological institutes is mainly aimed at ensuring the uniformity of measurements in quantitative analysis. In recent years, international cooperation has been developing in this area and international interlaboratory comparisons have been carried out. In quantitative analysis, it is of particular interest to determine the concentrations of components in the surface layers of samples from the Raman spectra.

At present, in Russia, random and systematic error of wave number, signal-to-noise ratio, spectral resolution; RMS deviation of the output signal are under control when testing and verifying Raman spectrometers. Polystyrene film and toluene or cyclohexane solutions, for which the wave numbers of
the main Raman peaks are known, are used as reference materials. Control of the relative or absolute intensity of the peaks is not provided during testing and verification. Therefore, the ratios between the intensities of different Raman peaks turn out to be instrument-dependent. This significantly complicates the comparison of Raman spectra measured on different instruments for the identification and classification of substances.

In order to ensure the uniformity of measurements in Raman spectroscopy, VNIIOFI begin research on improving the State primary standard of the unit of mass (molar) fraction and mass (molar) concentration of components in liquid and solid substances and materials based on spectral methods - GET 196. Within this improvement, a special module will be added to the standard, which will include a Confotec® NR500 Raman confocal laser microscope (manufactured by SOL Instruments, Belarus), see figure 1, and certified reference materials for calibrating Raman spectrometers and microscopes in relative intensity based on inorganic glasses activated with transition metal ions, and similar liquid measures based on cyclohexane.

![Figure 1. Raman confocal laser microscope Confotec® NR500.](image)

The glass-based reference materials will be 1.5 mm thick plates and are designed for scattering at an angle of 180°, they will be validated using a Confotec® NR500 microscope, for which the relative spectral sensitivity is calibrated using a reference light source traceable by the spectral irradiance to the radiometric standard. The accuracy on the wavelength scale is ensured by a low-pressure neon lamp included in the microscope, the spectrum of which is traceable to a database of atomic spectra. The use of these measures will ensure the instrument independence of the shape of the Raman spectra measured on working measuring instruments (Raman spectrometers and microscopes). The development will use the experience of VNIIOFI in creating fluorescence reference materials based on special glasses designed to reproduce fluorescence spectra [2].

The possibility of developing reference materials of confocal volume for Raman microscopes, necessary for determining the concentration of substances from the intensities of their Raman peaks, will also be investigated. The microscope will be able to excite Raman scattering at 532 nm, 633 nm, 785 nm and 1064 nm, the most used in analytical Raman spectroscopy. It is supposed to determine the mass fraction of components in the surface layers of solid objects and thin films in the range from 10^{-2} % with an expanded uncertainty not exceeding 10%.

In 2021 the metrological characteristics of Confotec® NR500 were investigated. It was found suitable for using this microscope as the base of the Raman module of the primary standard.

**Conclusion**

The implementation of the above program will provide the possibility of measuring instrument-independent Raman spectra, which will significantly expand the possibilities of Raman spectroscopy for qualitative analysis, including in the control of drugs and food products. Reference materials of confocal volume will allow using of Raman spectra for the quantitative determination of substance concentrations.
References

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[2] Sadagov Yu et al 2020 Spectral Standards Based on Glasses Activated with Rare-Earth Element Ions for the Calibration of Fluorescence and Raman Spectrometers *Optics and Spectroscopy* vol.128 (10) pp 1658-1666