Application of the shifted excitation Raman difference spectroscopy (SERDS) to the analysis of trace amounts of methanol in red wines

Boris L. Volodin,[a)] Sergei Dolgy,[a)] Vladimir S. Ban, [a)] Davor Gracin,[b)] Krunoslav Juraić, [b)] Leo Gracin[c)]

[a)] PD-LD, Inc. (United States)
30-B Pennington-Hopewell Rd. Pennington, NJ 08534

[b)] Rudjer Boskovic Institute (Croatia)
Division of Materials Physics, HR-10000 Zagreb, Bijenicka 54, Croatia

[c)] Department of Food Engineering, Faculty of Food Technology and Biotechnology - University of Zagreb, HR-10000 Zagreb, Pierottijeva 6, Croatia

ABSTRACT

Shifted Excitation Raman Difference Spectroscopy (SERDS) has proven an effective method for performing Raman analysis of fluorescent samples. This technique allows achieving excellent signal to noise performance with shorter excitation wavelengths, thus taking full advantage of the superior signal strength afforded by shorter excitation wavelengths and the superior performance, also combined with lower cost, delivered by silicon CCDs. The technique is enabled by use of two closely space fixed-wavelength laser diode sources stabilized with the Volume Bragg gratings (VBGs). A side by side comparison reveals that SERDS technique delivers superior signal to noise ratio and better detection limits in most situations, even when a longer excitation wavelength is employed for the purpose of elimination of the fluorescence. We have applied the SERDS technique to the quantitative analysis of the presence of trace amounts of methanol in red wines, which is an important task in quality control operations within wine industry and is currently difficult to perform in the field. So far conventional Raman spectroscopy analysis of red wines has been impractical due to the high degree of fluorescence.

INTRODUCTION

Raman spectroscopy has been experiencing a period of growing interest for qualitative and quantitative analysis in a vast scope of applications pertinent to various industries, including pharmaceuticals [1-5], petrochemical [6-8] and law enforcement [9-13]. This growth is enabled by increase in the offering of affordable Raman instrumentation, some is portable and ruggedized for field use. Such increase in availability and portability is powered in turn by compact, high-performance wavelength-stabilized laser diodes [14]. However, with widening of the scope of applications for Raman spectroscopy the challenge of sample fluorescence becomes more and more evident. Besides the natural fluorescence that many substances possess, there are issues of sample contamination with fluorescent compounds in the field that one must deal with in real life situations [9-10]. One example of that is fluorescent agents such as caffeine and flour regularly used cutting illicit street drugs [10].

One of the important classes of fluorescent substances are food products, of which red wine is one of the important examples. Raman analysis is very convenient for instant in-the-field measurements for process and quality control of the wine production. However, Raman analysis of red wine is greatly complicated by the presence of strong fluorescence (Fig. 1). For this reason, long-wavelength, typically1064 nm, Raman instruments, that are often considered the benchmark in dealing with fluorescent substances, have been marketed for use of red wine analysis [15]. However, dealing with long excitation wavelength presents a number of issues. First of all, as is well known, the Raman signal diminishes as the 4^{th} power of laser wavelength, which means that it is ~ 3.4 times weaker with 1064 nm excitation as compared with commonly used 785 nm excitation. Generally it means that significantly longer collection times are necessary to collect as much Raman signal when using 1064 nm laser. Furthermore, the use of 1064 nm excitation laser requires using InGaAs detectors instead of silicon CCDs. In case of dispersive Raman systems this means InGaAs arrays, which have more than an order of magnitude higher thermal noise than that of the silicon CCDs. As a result, InGaAs detector arrays for dispersive Raman instruments must be cooled to achieve satisfactory noise levels. Although cooling the detector is not a problem for laboratory instruments, it presents an obvious issue for portable, battery-operated Raman instrumentation, as it means more power consumption, larger battery and shorter battery life, in addition to increase in cost and complexity of the instrument.

For that reason a lot of attention has been paid over the years to other methods of combating fluorescence in Raman measurements, which can be separated into two classes: 1) the digital filtering methods; and 2) the physical methods of subtracting fluorescence contribution from the Raman signal, of which the Shifted Excitation Raman Difference Spectroscopy, or SERDS, is the prime example. SERDS approach is very attractive due to its ability accurately subtract fluorescence contribution regardless of its shape and particular spectral features. Thus SERDS can effectively expand the use of the conventional dispersive Raman instrumentation equipped with inexpensive and efficient CCD detectors to the classes of samples that exhibit fluorescence.

Historically the SERDS method has been used in laboratories employing tunable-wavelength lasers. However, the use of this type of laser sources presents a significant challenge for portable Raman systems. Generally these lasers are much more costly than simple and efficient wavelength-stabilized laser diodes. But in addition to this there is an issue of the exact wavelength control over these lasers required to perform accurate qualitative and especially

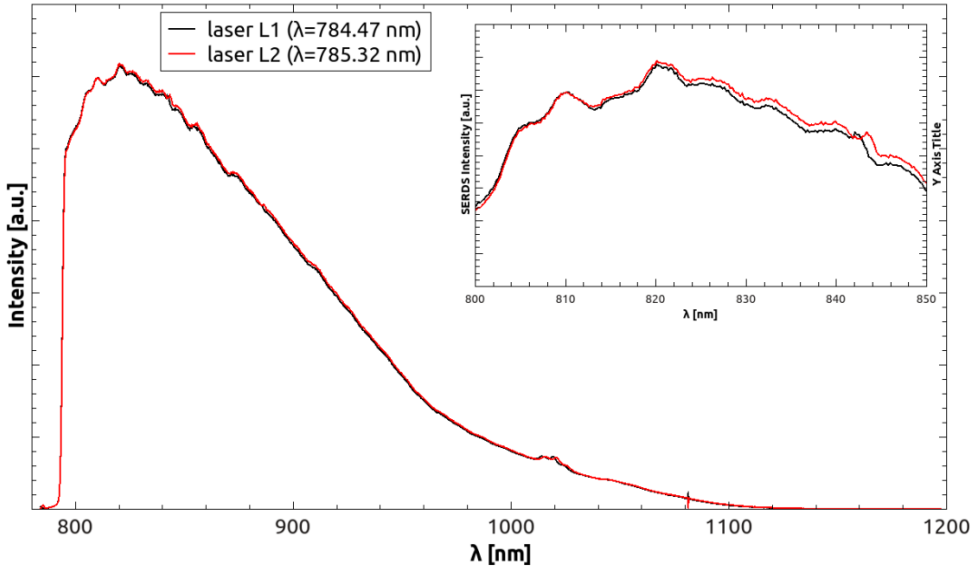

Figure 1. Raman spectra of a red wine collected with the two SERDS lasers with slightly offset laser wavelengths. The inset shows the spectral region from 800 to 850 nm in more detail.

quantitative Raman analysis, as it is not fixed or stable, which requires constant active wavelength monitoring for accurate analysis.

For these reasons we have investigating the use of a comparatively much more simple and practical approach to performing SERDS analysis using two affordable wavelength-stabilized laser diodes operating at slightly offset wavelengths. The lasers are stabilized by use of the Volume Bragg Grating (VBG) technology [16] and are very compact, efficient and inexpensive, making them well suited for portable battery-operated Raman instrumentation.

METHANOL IN RED WINE

Methanol is not a major constituent in wines. Within the usual range found in wine (0.1–0.2 g/liter), methanol has no direct sensory effect. Of the over 160 esters found in wine, few are associated with methanol. The concern occasionally voiced about methanol relationship to its metabolism into formaldehyde and formic acid. Both metabolites are toxic to the central nervous system. One of the first targets of formaldehyde toxicity is the optic nerve, causing blindness. Methanol never accumulates to toxic levels under legitimate winemaking procedures.

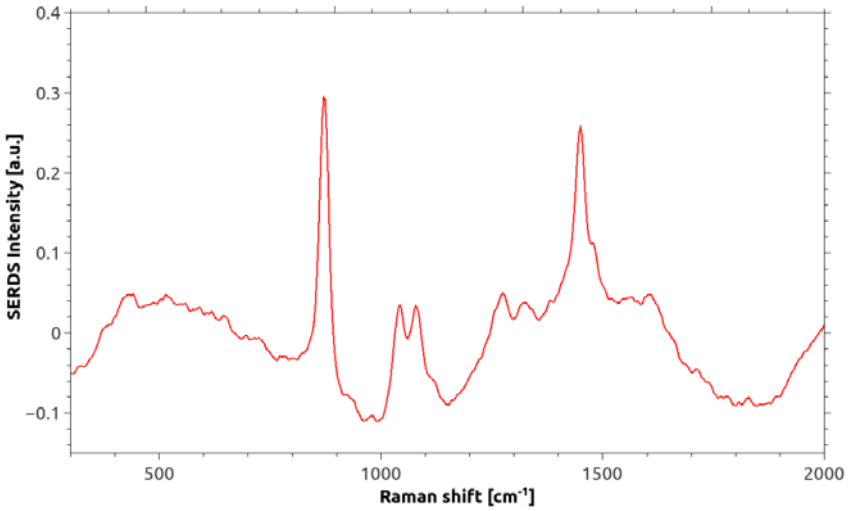

Figure 2. Raman spectrum of red wine reconstructed from SERDS difference spectrum obtained using the raw Raman spectra shown in Fig. 1.

The limited amount of methanol found in wine is primarily generated from the enzymatic breakdown of pectins. After degradation, methyl groups associated with pectin are released as methanol. Thus, the methanol content of fermented beverages is primarily a function of the pectin content of the substrate. Unlike most fruits, grapes are low in pectin. As a result, wine generally has the lowest methanol content of any fermented beverage. Pectolytic enzymes, added to juice or wine to aid clarification, can inadvertently increase the methanol content. Adding distilled spirits to a wine also may slightly increase the methanol content. The concentration of ethanol and other flavor compounds achieved with distillation augments the methanol content of the distillate.

SHIFTED EXCITATION RAMAN DIFFERENCE SPECTROSCOPY

The underlying principle of the SERDS is the fact that Stokes and Anti-stokes components of the Raman scattering are frequency-shifted relative to the excitation wavelength and, therefore, are tied to it. Laser-induced fluorescence, on the other hand, is independent from the excitation wavelength and retains practically identical spectral shape when the excitation wavelength experiences a relatively small change (< 10 nm, for example). For that reason if two spectra are collected with a small shift in the excitation wavelength and subsequently subtracted, the resultant spectrum will be free from the fluorescence contribution, while retaining the Raman signal, albeit in a form of a difference spectrum.

The most important advantage of the SERDS method over the conventional methods of digital filtering of the fluorescence background is accurate elimination of the fluorescence contribution, as opposed to approximation of such using a polynomial function, as is the case in baseline fitting, for example. This advantage is particularly obvious when the background fluorescence has some spectral features, but even in the situations when the fluorescence has a slowly varying smooth shape the advantages of SERDS are very apparent.

Using two fixed wavelength VBG-stabilized laser sources is a particularly simple and efficient method of implementation of SERDS [17-19]. Such sources are now available in high brightness and high power at a variety of wavelengths, and are very reliable and stable in wavelength over the lifetime of a device. These lasers do not require constant wavelength monitoring as their wavelength is known and stable.

Availability of such sources in a wide range of spectrum, from the 400 nm to the near IR, is another convenient feature, which allows the end user the freedom of choice of the excitation wavelength. Since these laser sources are compact, they are very compatible with field-portable Raman instruments, which presents an important advantage, as the signal collection in the field is often not free from background light and the samples are often not pure, thus increasing the likelihood of encountering interference from the fluorescence of the contaminants.

Being able to analyze fluorescent samples while using the preferred, i.e. shorter, excitation wavelength has the advantage of being able to use silicon CCD detectors, which have high sensitivity, lower thermal noise and low price tag associated with them. Furthermore, SERDS can be implemented without any changes to the Raman probe and spectrometer hardware, making it simple and versatile for equipment manufacturers. When equipped with silicon CCD, even a portable Raman instrument will have higher resolution and faster signal processing times then laboratory-size long-wavelength Raman instruments. Because the Raman signal is so much stronger at shorter wavelengths the signal collection times are often many times faster for 785 nm systems, for example, as compared with the 1064 nm system for similar laser irradiance impingent on the sample.

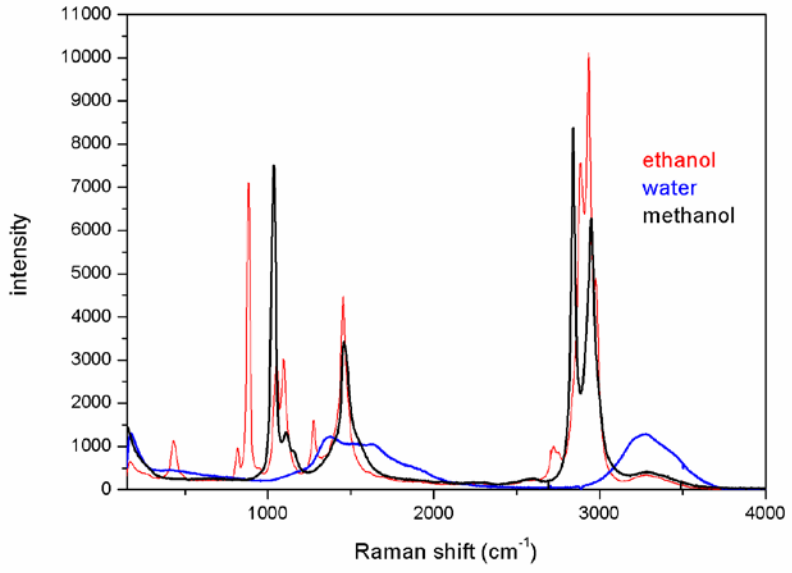

Figure 3. Raman spectra of the major constituents of the phantom wine used for calibration of the method.

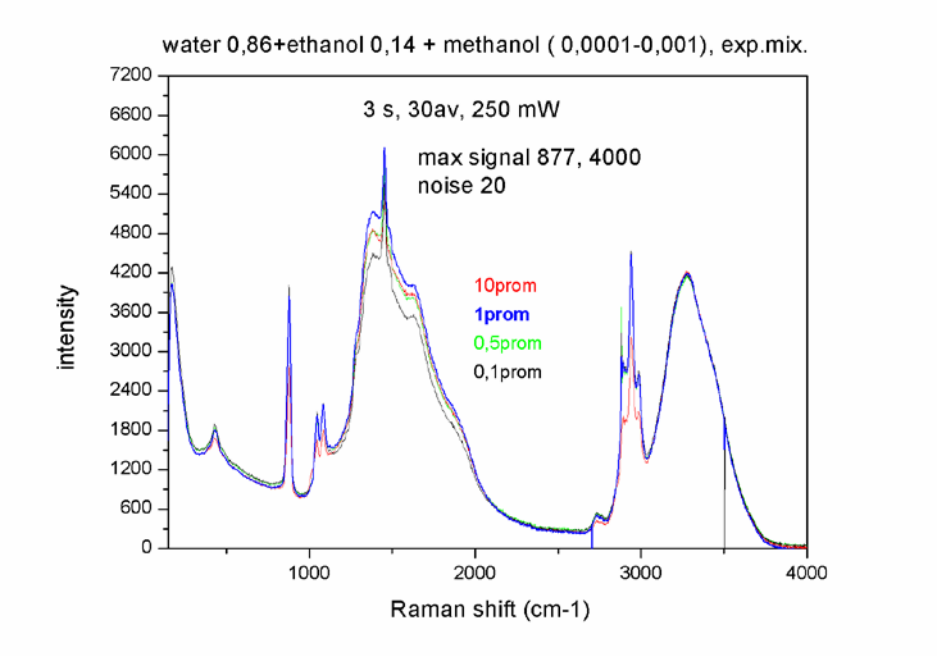

Figure 4. Raman spectra of phantom wines with different amount of methanol added.

As the signals for the two excitation wavelengths are collected in rapid succession, the SERDS method does not require collecting the background signal independently, as it is automatically corrected for it during the subtraction of the two collected spectra.

The main objection against the SERDS method that has been often voiced concerns the fact that in subtracting the fluorescence contribution from the measured spectrum its shot noise not only remains but also gets increased by adding the noise from the second spectrum. And the fluorescence shot noise will increase proportionately to the square root of the fluorescence intensity. While that is true, what is typically not taken into account that shot noise will be reduced if multiple spectra are averaged. The fact is that with increasing fluorescence the exposure/integration time of the CCD has to decrease inversely proportionately to its intensity, thus allowing for increasing the number of averages within the same total measurement time directly proportionately to the fluorescence intensity. As a result, the shot noise will decrease proportionately to the inverse of the square root of the number of averages. Therefore, the resultant SNR for SERDS measurement should be *independent of the fluorescence shot noise* when the total measurement time remains about the same:

$$SNR \sim \frac{I_R}{\sqrt{I_F}}\sqrt{N} \sim \frac{I_R}{\sqrt{I_F \Delta t}} \sim \frac{I_R}{\sqrt{I_F}}\sqrt{I_F} \sim I_R, \qquad (2)$$

where I_R and I_F are the Raman and fluorescence intensities, respectively, N is the total number of averaged spectra, and Δt is the integration time for a single spectrum. Therefore, it should be possible to keep the SNR of SERDS measurements nearly free from the influence of the fluorescence shot noise.

EXPERIMENTAL

The SERDS experiments were performed using a dual-laser SERDS laser source, LS-2, produced by PD-LD, Inc. The lasers operated at the 784.5 nm and 785.5 nm wavelengths. The wavelength separation was selected to correspond to the

approximate line width of the Raman lines of the substances under study. Note that the exact wavelength separation is not significant for the accuracy and practicality of SERDS, however, wavelength separation that is much smaller than the width of the Raman bands would result in increase in noise in SERDS spectra.

The LS-2 laser source delivers the output of either one of the lasers to the output port via a fiber-optic switch. A fiber-optic cable Raman probe was attached to the output port of the LS-2. The probe delivered the laser light to the sample and then collected the Raman signal in the back-reflection geometry, together with fluorescence and the Rayleigh scattering. The notch filters installed in the Raman probe suppressed the Rayleigh scattering and the collected signal was delivered to the input port of the spectrometer via the collection fiber in the probe.

For the experiments with 785 nm excitation we used Ocean Optics Maya spectrometer. The spectrometer had 50 um slit and approximately 10 cm^{-1} resolution. The detector was an uncooled back-thinned CCD with 2048 pixel array from Hamamatsu. For SERDS analysis the spectra were collected sequentially with laser #1 and then with laser #2. The lasers ran continuously during the course of the experiment to assure best stability of the output power. The wavelengths of the lasers were stable to < 5 pm over the course of the day. Majority of the samples were placed in small glass vials and illuminated through the bottom of the vial.

The collection of spectra for SERDS was done with identical power on the sample for both laser sources. Nevertheless, the obtained spectra were normalized to the full integral of a spectrum within the selected spectral band of interest (the region near the laser wavelength that is suppressed by the laser rejection filter was excluded from the integration, for example) before the subtraction. This procedure ensured that there was no bias in the data due to different total signal intensities collected with the SERDS laser pair. As a result, all SERDS spectra had zero mean value. Furthermore, in order to produce data that are free from the measurement artifacts the data were corrected for the white light response function (this procedure is also called flat-field correction when performed on camera focal plane arrays).

The experiments were performed with about 300 mW of optical power impingent on the sample for either of the two SERDS lasers. The exposure time was always selected to keep the maximum of the signal just below the saturation

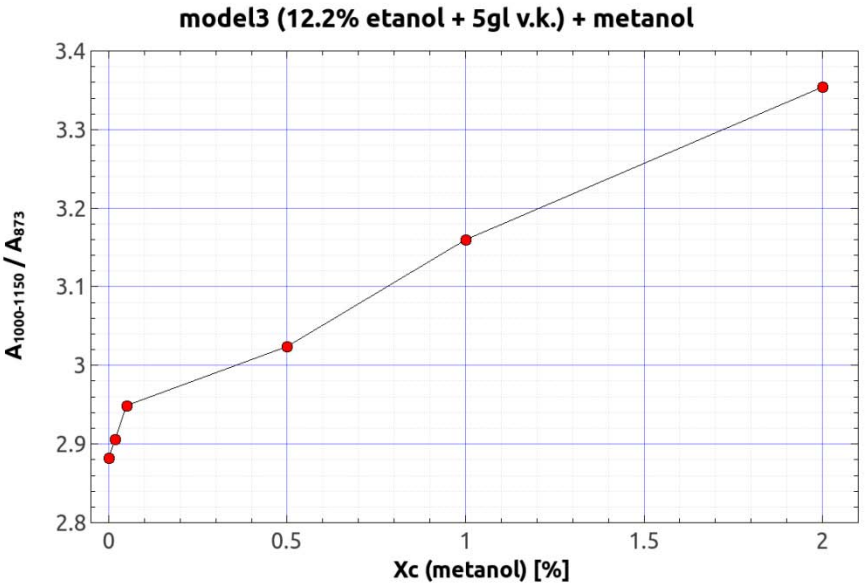

Figure 5. Calibration curve for the determination of the methanol content in phantom wine (water + 12.2% ethanol + 5 g/l tartaric acid).

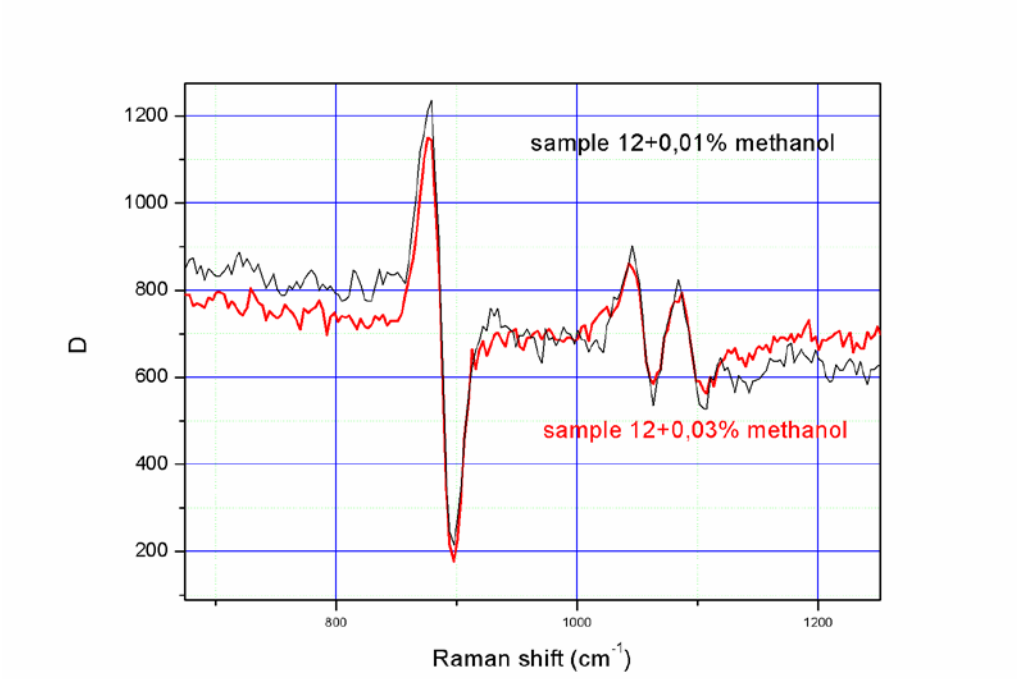

Figure 6. Differential SERDS spectra of red wine with different methanol content.

level of the detector array. Therefore, when large amount of fluorescence was collected the exposure time was shortened accordingly. When performing analysis of highly fluorescent samples, we have employed signal averaging to increase the signal to noise ratio. Note that due to the shortening of the exposure time when fluorescence is present there can be a proportionately larger number of averages performed within the same measurement time. Furthermore, due to smaller Raman scattering cross-section at longer wavelength, the measurement times for the 1064 nm system are typically longer than those for the 785 nm systems (approximately by factor of 5 for the systems employed in this study).

All collected spectra were treated according to the same procedure. Once the Raman spectrum of the sample was obtained and saved, the resultant data were corrected by the combined spectral response of the system, which included the transmission of the laser suppression filters and the relative pixel response of the CCD. Therefore, all the spectra used in this study were free from the stationary noise (e.g. pixel noise of the detector).

Note that in the results presented in this paper the SERDS spectra were reconstructed using a simple integration with data interpolation algorithm [20] in order to present the spectra in a conventional form. However, it is not generally required to perform the spectrum reconstruction and excellent results can be obtained using the differential SERDS spectra.

RESULTS

Raw spectra of red wine collected with the two SERDS lasers is shown in Figure 1. The inset shows the details of the spectral region between 800 nm and 850 nm. Large amount of fluorescence is clearly evident. When SERDS method is applied to the same spectra with subsequent spectrum reconstruction, a spectrum similar to the one shown in Fig. 2 is obtained. In the reconstructed spectrum the prominent spectral features of the ethanol present in wine are now clearly

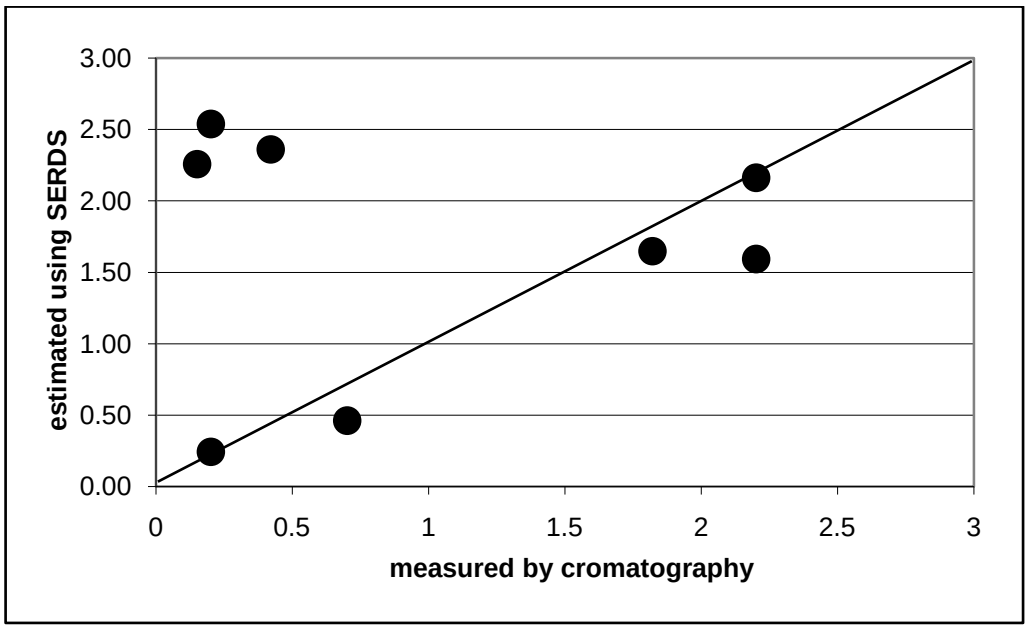

Figure 7. Comparison of the prediction of methanol content in red wine by SERDS with gas chromatography. In SERDS method reconstructed spectra were used as described in the text. The concentration of methanol is shown in promils (1 promil = 0.1 %).

resolved. Note that the reconstructed spectrum contains a wavy baseline that is the artifact of the reconstruction algorithm. For that reason quantitative analysis based on differential spectra can be much more accurate.

For the calibration of the quantitative analysis method of the methanol content in wine a model phantom mixture of wine was prepared. The main components of the wine phantom are water, 12% ethanol and methanol in various concentrations. The Raman spectra of these individual pure components are shown in Fig. 3. The spectrum of a mixture of 14% of ethanol+86% water and traces of methanol (0.001-0.01 volume %) are shown in Fig 4. For the quantitative analysis of small amounts of methanol in the mixture with ethanol, the best spectral features are the peak around 1000 cm^{-1} for methanol and the one around 850 cm^{-1} for ethanol.

Raman spectra of the phantom wines, i.e. the mixture of water, ethanol and methanol in various concentrations are shown in Fig. 4. The changes in spectra resultant from the addition of trace amounts of methanol are small. The aim of this study is to detect traces of methanol, i.e. it is expected to be able to measure less than legal limit, which is in the 0.02-0.05 % range. If looking only at the intensities of the spectral peaks, the signal of methanol in actual mixture could be on the order of a few CCD counts. The noise of the signal, therefore, has to be well below, which can be achieved by averaging of a large number of scans. Typical value for averaging of 400 scans can be in the 2-3 counts range, which limits the sensitivity to about 0.05-0.1% of methanol. Some improvement can be obtained if integral intensity of the two parts of spectra are used for comparison, instead of just the peak intensities. Typically we compared the integral of the region between 950 and 1200 cm^{-1} with integral under the spectral peak around 850 cm^{-1}. Two times higher sensitivity can be obtained when comparing the intensities under the peaks around 850 and 1060 cm^{-1}, after fitting this region with 2 Voight profiles, with the peaks at 1060 and 1100 cm^{-1}, since changes in methanol concentration affect more the first one than whole interval. The results of the calibration of the method using the wine phantoms are shown in Fig. 5.

When red wine is analyzed, the fluorescence contribution is very strong, which results in the maximal signal for 14% ethanol to be around 1000 counts, as is seen in the SERDS spectrum shown in Fig 6. Consequently, the main

methanol peak for 0.05 % of methanol content results in ~ 3 CCD counts, which is in the range of noise, and, therefore, requires employing spectral averaging. When 400 spectral averages were performed we were able to detect 0.05 % of methanol in red wine. This result was verified by gas chromatography (Fig. 7). The most critical point was estimation of the base line which affects the results of fitting and consequently the calculated amounts of methanol. Therefore, using differential spectra combined with multivariate calibration methods are expected to yield better results in the future.

CONCLUSIONS

As is evident from these results, the SERDS method allows detection of methanol in red wine below the legally allowed limit. Its advantage in comparison with the baseline fitting method is primarily in accurate elimination of the fluorescence, whereas the latter method allows only approximate removal of the fluorescent background and only in the situations where it is varying very slowly. Furthermore, the method employed for red wine analysis so far utilized the Raman reconstructed from SERDS signal, which also suffers from the residual artificial baseline. Further improvements in the detectable limits of the methanol are expected when multivariate methods, such as partial least squares regression, or PLSR, are applied to the differential SERDS spectra directly.

Overall, the advantages of higher resolution, larger number of pixels, better thermal noise and lower cost of the silicon CCDs make the 785 nm Raman systems very attractive for the field use. Therefore, it is quite clear that the availability of SERDS method made possible by the use of the VBG technology offers clear advantages for many situations where fluorescence is an impediment to Raman analysis.

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