Title

The self-absorption phenomenon in quantitative Raman spectroscopy and how to correct its effects

Authors

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ABSTRACT

When the wavelength of the Raman scattered light coincides with the absorption spectrum of the sample, a phenomenon known as Raman self-absorption occurs. This process can causes spectra with low intensity / noise ratios, and/or produce modifications of the band profile of the Raman bands that are difficult to be detected. If it is not possible to use another laser excitation source in order to avoid this phenomenon, these modifications must to be corrected. Nowadays, the known correcting methods have been developed with the aim of obtaining quantitative analysis of the samples *i.e.* for knowing concentrations, are very complicated and require the knowledge of several parameters.

In this work, we present a very simple approach based on the Lambert-Beer law with the aim of correcting the modifications of the band profile due to the low self-absorption. This method uses simple concepts, a direct work methodology and to apply it, it is not necessary to know any type of sample parameter except the concentration of the Raman active component in the liquid matrix. The developed approach is applied to Raman spectra of $CuSO_4$ aqueous solution with satisfactory results.

Keywords: Self-Absorption, Raman spectra, Decay of intensity correction.

INTRODUCTION

Raman spectroscopy is considered one of the most promising qualitative techniques in many scientific fields, as it provides not only information about the molecular atomic structure of almost any material, but also yields information about the environment surrounding molecules [1,2,3]. Besides, Raman spectroscopy can be used as a quantitative technique, inasmuch as of the Raman signal intensity depends (among other parameters) on the concentration in the solution [4]. This allows monitoring chemicals concentrations in chemical reactions, catalytic processes [5,6], and countless other applications [7,8].

Several processes can coexist with the Raman effect and thus affect the resulting spectra, such as fluorescence. While fluorescence has been widely studied, self – absorption is one of the processes which have been less studied. This phenomenon occurs when the wavelength of the Raman scattered light overlaps with the absorption spectrum of the sample [9, 10]. If the absorption coefficients of the sample are high in the wavelength range of the Raman spectrum the sample may absorb the laser excitation radiation as well as the Raman scattered light, therefore the acquired spectrum will have low quality (low intensity / noise ratio), or even undetectable Raman signal. A tricky case may occur if the absorption coefficients of the sample are rather small resulting in modifications of the Raman spectrum difficult to identify: this can occur when the tail of the absorption spectrum coincides with the Raman spectrum range for a given laser excitation line. In addition, as the absorption coefficient is wavelength dependent, the spectrum obtained is not only less intense, but may produce modifications of intensity ratios between the different bands and modify the band profile of Raman bands with large width.

The easiest way to avoid this phenomenon is to change the excitation line in order to produce a Raman scattering signal not coincident with the absorption spectrum of the sample. If another excitation line is not available, the intensity of the Raman spectrum must be corrected in addition to the intensity corrections carried out for the whole spectrometer.

Despite the importance of this phenomenon in Raman spectrometry only a few authors have attempted to correct it. Ludwig *et al.* [11] quantified the intensity losses with a numerical approach for a macroscopic illumination-collection light cell with a 90° Raman scattering geometry. Their method is based on some geometrical requirements, such as a cylindrical container, and uses complex equations. Another interesting approach was done by N. Erewall [12] who studied the self-absorption in Near-Infrared Fourier transform Raman spectroscopy in a confocal set-up in a 180° collecting geometry.

Lin L.-T. et al.[13] developed a method for a confocal set-up and 180° backscattering collection geometry. This method compares the absorption in the studied sample with a standard, calculating a correction factor. Using this approach Z. Wu et al.[14] studied the self-absorption where the molar absorption coefficient of the sample and standard are very similar for both liquid and solid systems. In this case the correction factor for the band can be easily calculated. This has also been successfully applied by J.H. Hooijschuur et al. [15]. Nevertheless, it is important to note that finding a standard reference with similar absorptivity coefficients as the studied sample is not always possible, especially dealing with broad bands.

The described methods are meant to use Raman spectroscopy in quantitative analysis. In this study, the solution concentration is a known parameter. As previously say self-absorption may affect some parts of the Raman spectrum more than others and modify the expected profile. In this paper we present a simple method, based in the Beer-Lambert law, for correcting the self-absorption in Raman spectra of liquid and solutions. The method is based in the analysis of spectra of a solution of a given concentration with different absorption. Solutions of $CuSO_4$ were used to verify the effectiveness of this method.

MATERIALS AND METHODS

Copper sulfate penta-hydrate ($CuSO_4 \cdot 5H_2O$) was purchased from VWR, International, and re-crystallized before being used. Milli-Q water, with total organic carbon lower than 5-10 ppb and resistivity higher than $18 m\Omega\ cm^{-1}$ was used as a solvent to prepare four solutions with the following concentrations: 0.28, 0.40, 0.71 and 0.87 M. Magnesium sulfate hepta-hydrate ($MgSO_4 \cdot 7H_2O$) for preparing 0.4 and 0.85 M magnesium sulfate solution, was purchased from SIGMA-ALDRICH (pro analysis ≥99%), and used without any previous purification processes.

Most experiments were carried out, using a Raman Confocal microscope (Voyage^{TM} BWS435-532SY), coupled with an Olympus BX51 microscope. We collected the scattered light in a backscattering configuration using a 20 x long working distance, NA 0.40 objective mounted on the microscope. The excitation line was provided by a Nd-YAG solid state laser with narrow band of nominal wavelength 532 nm. The spectrometer has a unique monochromator double path model BWS435 coupled to a CCD detector model Hamamatsu S10141-1107S. The Raman spectral range is from 84 cm^{-1} to 3750cm^{-1}. The pixel resolution is ~ 4 – 5 cm^{-1}/pixel. For optical observation a Chameleon^{TM} CMLN-13S2C digital camera has been coupled with the equipment. The typical exposition time was 20 s and the number of accumulations 16, each spectrum has been taken three times. In comparing harves, a Raman equipment JASCO NRS4100 with a laser excitation source of 457 nm have been used on a one-off basis. The analysis of the data has been carried out with commercial software, OriginPro8.

In order to obtain spectra of the same sample with different absorptions, spectra at different path length, *l*, are acquired. The optical path is the distance from the surface of the solution to the focus point from which the spectrum is acquired, *i.e.* the depth. Thus, with increasing optical path length, the absorption increases. For each spectrum l, of the focal point is carefully controlled using a home-made cell. A very simple cell was designed to acquire the spectra of the solutions as function of the optical path length, which is the distance from the surface of the solution to the focal point from which the spectrum is acquired (*i.e.* the depth). Thus, with increasing optical path, the absorption increases. The main purpose of this cell is to minimize the water meniscus of the water solution, because this meniscus can provoke errors in measuring depth of the focusing point of the microscope respect to the surface of the solution. To achieve this purpose the cell has a soup plate shape with a 6 cm diameter and 2 cm high. Inside this cell a 3 mL solution has a high of 7 mm.

The absorption spectrum of an aqueous copper solution 0.4 M have been obtained using a Jasco model V-530.

RESULTS AND DISCUSION

Raman spectra of $MgSO_4$ and $CuSO_4$ solution (C=0.4M) obtained at the same depth, 1.2 mm, can be divided into four zones (Fig. 1.A): i) the low wavenumber region between 100 - 700 cm $^{-1}$ assigned to H_2O translation band [16], ii) a second region with only a narrow and intense band around 984 cm^{-1} (Fig. 1.B) corresponding to the S-O symmetric stretching [17], iii) a third region corresponding to intramolecular vibrational bands [18] of water molecules in the liquid state *i.e.* the H_2O bending mode~1500-1750 cm^{-1}, and iv) a broad band from 2750 cm^{-1} to 3750 cm^{-1} corresponding to the OH-bond stretching, (Fig 1.C).

The intensity of the Raman bands of a solute in a solution, I_R, is proportional to the concentration [*e.g.* 19], thus:

$$I_R = (I_L \sigma \eta d)\, C \qquad \text{Eq.1}$$

where I_L is the laser intensity, σ is the Raman cross-section or scattering efficiency, η is an instrument intensity correction parameter, d is the sample path length, and C is the concentration in the molarity scale. Therefore relative intensities, which are independent of optical path, exposure time, and other experimental factors, may be used to determine relative concentrations. A typical procedure to compare band intensities is to normalize each spectrum with a known constant feature in all spectra to be compared. In case of water solution spectra a good choice as common feature is the intensity of the isosbestic point of water located at 3468 cm^{-1} [20] (Fig. 1.C). Intensity normalized Raman spectra of the S-O stretching band (I_{S-O} / I_{3468}) (Fig. 1.B) of the $MgSO_4$ and $CuSO_4$ solutions is higher for the $CuSO_4$ solution than the one of the $MgSO_4$. As both solutions have the same concentration this behavior could be explained by the decrease of the intensity at the isosbestic point by a self-absorption process.

This can be proved in Figure 2, were a Raman spectrum of a $CuSO_4$ solution has been compared with an absorption spectrum of the same solution. One can see the Raman scattering of the water band (O-H stretching) in the 2700 – 3750 cm^{-1} region, using an excitation line of 532 nm, occurs in a spectral range where the absorption of the $CuSO_4$ solution is increasing. Only the tail of the absorption spectrum coincides with the Raman scattering of the O-H stretching bands. However, the decay of Raman intensity is important enough to provoke distortions in the Raman spectrum.

At this point is clear the existence of self-absorption phenomena on the $CuSO_4$ aqueous solutions, and the possible effects that can provoke in a spectrum have been shown. In the following part the correction method developed in this paper is explained:

The quantification of light absorption is modelled with the Beer-Lambert law [21].

$$\frac{I(\nu)}{I_0(\nu)} = 10^{\varepsilon\,(\nu) d \cdot C} \qquad \text{Eq. 2}$$

where $I_0(\nu)$ and $I(\nu)$ are the intensities (photon numbers) of the irradiating radiation (ν) and transmitted radiation (ν), $\varepsilon(\nu)$ the absorption molar coefficient, C is the molar concentration of the solute and d is the optical path.

The ratio of Raman scattering intensities at depth 0 and d calculated from equation 2:

$$\frac{I_0^*(\tilde{\upsilon}_0 - \tilde{\upsilon}_s)}{I(d, \tilde{\upsilon}_0 - \tilde{\upsilon}_s)} = \frac{1}{exp^{[-\varepsilon(\tilde{\upsilon}_0 - \tilde{\upsilon}_s) \cdot C \cdot d]}} \qquad \text{Eq. 3}$$

where $I_0^*(\tilde{\upsilon}_0 - \tilde{\upsilon}_s)$ is the Raman intensity in the absence of absorption by the solution which depends on the difference between the laser radiation energy $\tilde{\upsilon}_0$ and the energy of the vibration $\tilde{\upsilon}_S$. $I(d, \tilde{\upsilon}_0 - \tilde{\upsilon}_s)$ is the Raman intensity at $\tilde{\upsilon}_0 - \tilde{\upsilon}_s$ of the solution collected at depth d, the optical path length of the exciting and Raman radiation. $\varepsilon(\tilde{\upsilon}_0 - \tilde{\upsilon}_s)$ is the molar absorption coefficient of the solution at $(\tilde{\upsilon}_0 - \tilde{\upsilon}_s)$ Raman shift for the $\tilde{\upsilon}_0$ excitation line.

Our method consists in obtaining the variation of the molar absorption coefficient as a function of the Raman shift for a given solution, $\varepsilon(\tilde{\upsilon}_0 - \tilde{\upsilon}_s)$. When this function is obtained and, if the parameters, C and d are known, the application of Equation 3 allows us to correct any spectra taken at any optical length.

The experimental procedure for obtaining $\varepsilon(\tilde{\upsilon}_0 - \tilde{\upsilon}_s)$, consists in three simple steps. First, spectra of the $CuSO_4$ 0.4 M solution are collected at different path lengths (Figure 3). The optical path length is the distance from the surface of the solution to the focus point from which the spectrum is acquired. With increasing optical path, Raman spectra show a strong intensity decrease of the stretching band of water whereas the symmetric stretching vibrational band of sulfate remains constant.

The second step consists to calculate $I(0, \tilde{\upsilon}_0 - \tilde{\upsilon}_s)/I(d, \tilde{\upsilon}_0 - \tilde{\upsilon}_s)$, where $I(0, \tilde{\upsilon}_0 - \tilde{\upsilon}_s)$ is the intensity at the surface, *i.e.* d=0; Then we plot this ratio versus the Raman shift (Fig. 4). Such data show an increase of this ratio versus optical path. Then, the molar absorption coefficient, $\varepsilon(\tilde{\upsilon}_0 - \tilde{\upsilon}_s)$ is obtained from the linearization of Equation 3 by using logarithms (Equation 4):

$$log\left(\frac{I(0,\tilde{\upsilon}_0-\tilde{\upsilon}_s)}{I(d,\tilde{\upsilon}_0-\tilde{\upsilon}_s)}\right) = \varepsilon(\tilde{\upsilon}_0 - \tilde{\upsilon}_s) \cdot C \cdot d \qquad \text{Eq. 4}$$

Values of $log\left(\frac{I(0,\tilde{\upsilon}_0-\tilde{\upsilon}_s)}{I(d,\tilde{\upsilon}_0-\tilde{\upsilon}_s)}\right)$ plotted versus d at 3000, 3250 and 3410 cm^{-1} (Figure 5) with respect to the 532 nm exciting radiation are linear in accordance with equation 4 derived from the Beer-Lambert law. The absorption molar coefficient at each wavelength (Raman shift with respect to 532 nm excitation) is derived from the solution concentration and the slope of fitted straight lines, (0.0193 with an r^2 value of 0.99762 for 3000 cm^{-1}; 0.01429 with a r^2 value of 0.99886 for 3250 $cm^{-1}$and 0.416 with a r^2 value of 0.99959 for 3410 cm^{-1}).

The molar absorption coefficient for $CuSO_4$ 0.4 M solution versus the Raman shift under 532 nm excitation is obtained with the procedure shown above (Figure 6).

The corrected spectrum for a given known concentration acquired at known depth *d* are calculated from Raman spectrum obtained at zero path length using equation 4 and the experimental values of the extinction coefficients versus Raman shift (Fig. 6).

It should be important to note that the self-absorption process changes the OH-stretching vibrational band profile. In Figure 7, it is shown a comparison of a band profile of the OH-stretching bands of pure water, 0.85 M $MgSO_4$ and 0.85 M $CuSO_4$. As it can be seen the shape of this band corresponding to the $CuSO_4$ solution is slightly different. The water symmetric stretching band can be considered to result from three main different contributions [1] as the result of different interactions that the central H_2O molecule in a 5 molecule cluster with its neighbor H_2O molecules. In Figure 7 these contributions are indicated as follow: (1) indicates the contribution for water clusters (multiple), (2) corresponds to simple OH bonds and (3) corresponds to not hydrogen bonded OH bonds. The differences in the contribution bands of the spectra can be easily recognized. In the spectra of pure water (Fig. 7.A) and $MgSO_4$ (Fig.7.B), the contribution bands are pretty similar. Contrary, the contribution of these bands in the $CuSO_4$ spectrum does not follow the same behavior, *i.e.* the most important contribution seems to correspond to the water clusters (1) and the band of the non-bonded OH-Bond (3) is almost negligible.

Although, the distorted shape of the O-H stretching band can be explained by the self-absorption phenomenon, we have also compare the spectrum of $CuSO_4$ and $MgSO_4$ by using other laser excitation source of 457 nm, in order to test if the change profile could be the result from cation-water interactions (*i.e.* Cu-hydration). Figure 8 shows this comparison of the OH-band profile of the spectra obtained from $CuSO_4$ and $MgSO_4$ 0.4M and also shows the contributions bands of each spectrum which are very similar probing that there is not effect of the Cu-hydration.

Once, we had analyzed the phenomena and explained in detail the methodology, let´s apply the approach for correcting the self-absorption contribution to $CuSO_4$. The spectra of a 0.87 M $CuSO_4$ solution acquired at two different depths (d= 0.97 and 1.53 mm), are shown in Fig. 9.A, together with the spectrum obtained at the solution surface (d=0 mm) for the sake of comparison. The corrected spectrum for each depth are given in Figure 9.B, these spectra are identical at both depths which validates our approach. Thus, the Raman self-absorption correction is based easy to correct: it is based on the depth change in intensity of a given Raman signal as a function of the depth of the optical path onto the solution.

In addition, we had confirmed the three main contributions of the band profile of the OH-stretching before (Fig. 9.C) and after (Fig.9.D) applying this correction.

The interesting point of this methodology is that it is not necessary to measure independently the absorption coefficient by a UV-Vis absorption spectrometer. Moreover, this simple method require only the knowledge of the concentration, making it a convenient method to correct the change of intensity in of liquid samples.

CONCLUSIONS

This paper is focused on the correction of Raman band shape potentially spectrum which is modified by the self-absorption process. The method is based on the Beer-Lambert law to derive experimentally the self-absorption coefficient at each Raman shift by measuring the intensity of the Raman signal versus the depth of the optical path in a solution of a known concentration. The method has been developed and checked using aqueous solutions of $CuSO_4$ with satisfactory results.

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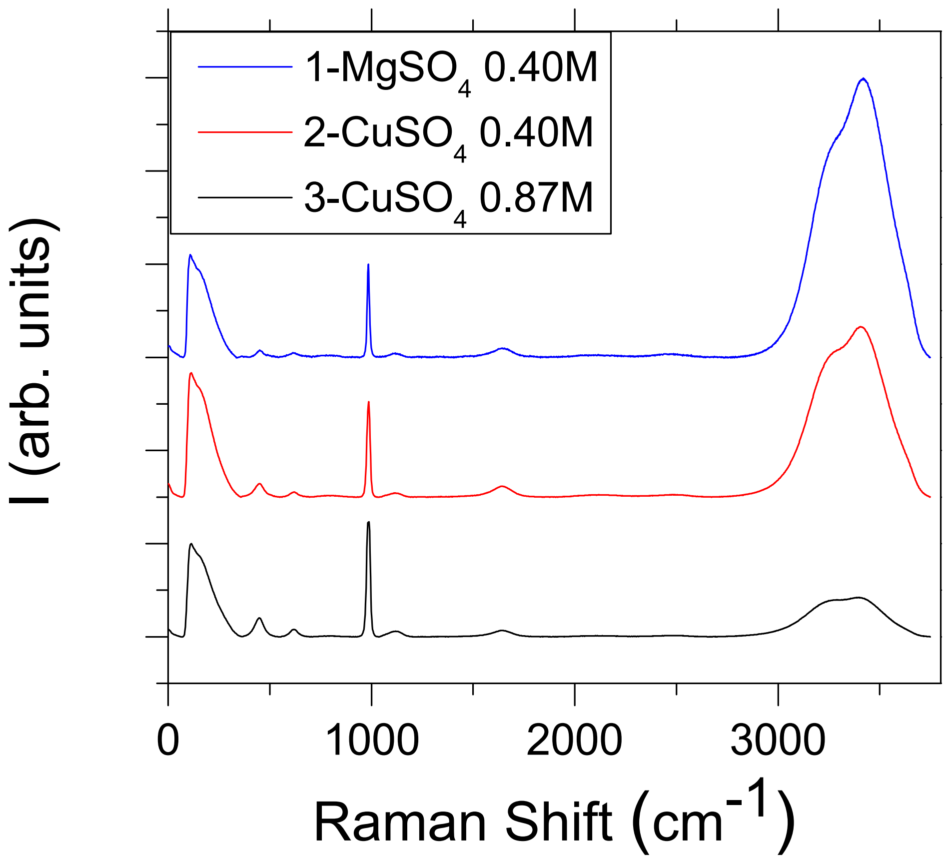

Figure 1. Raman spectrums from aqueous solutions of (1) $CuSO_4$ 0.87M (2) $CuSO_4$ 0.4M and (3) $MgSO_4$ 0.4M.

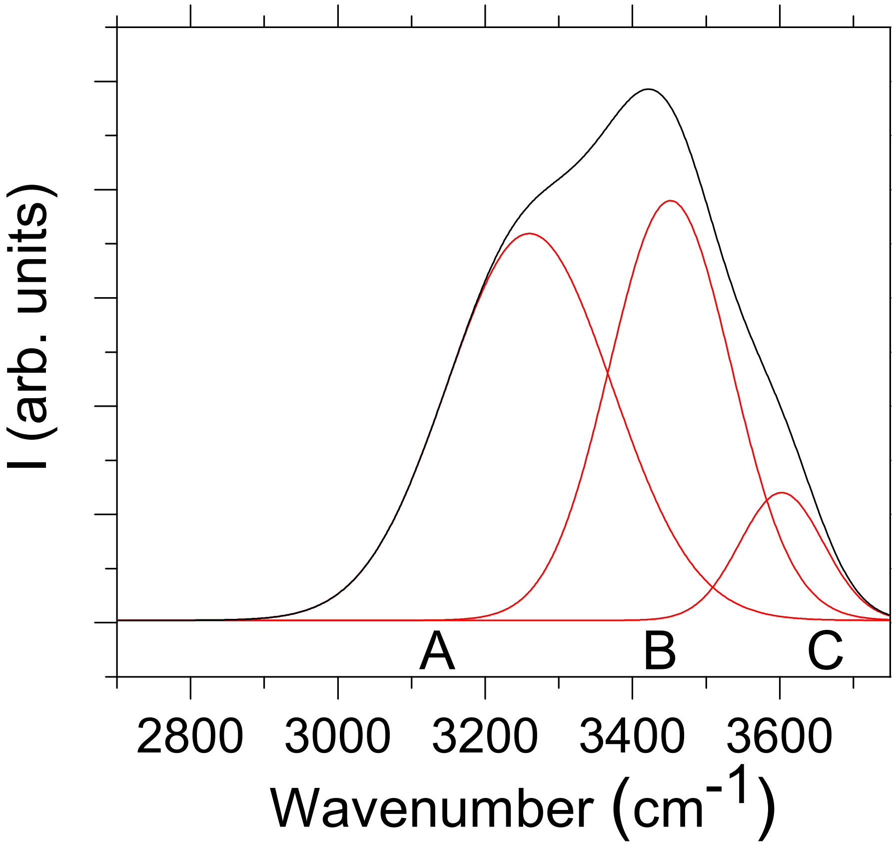

Figure 2. Raman spectrum for Milli-Q water, were the three contribution are shown, A is the contribution for water clusters, B correspond to chains of water molecules and C correspond to not bounded molecules of water.

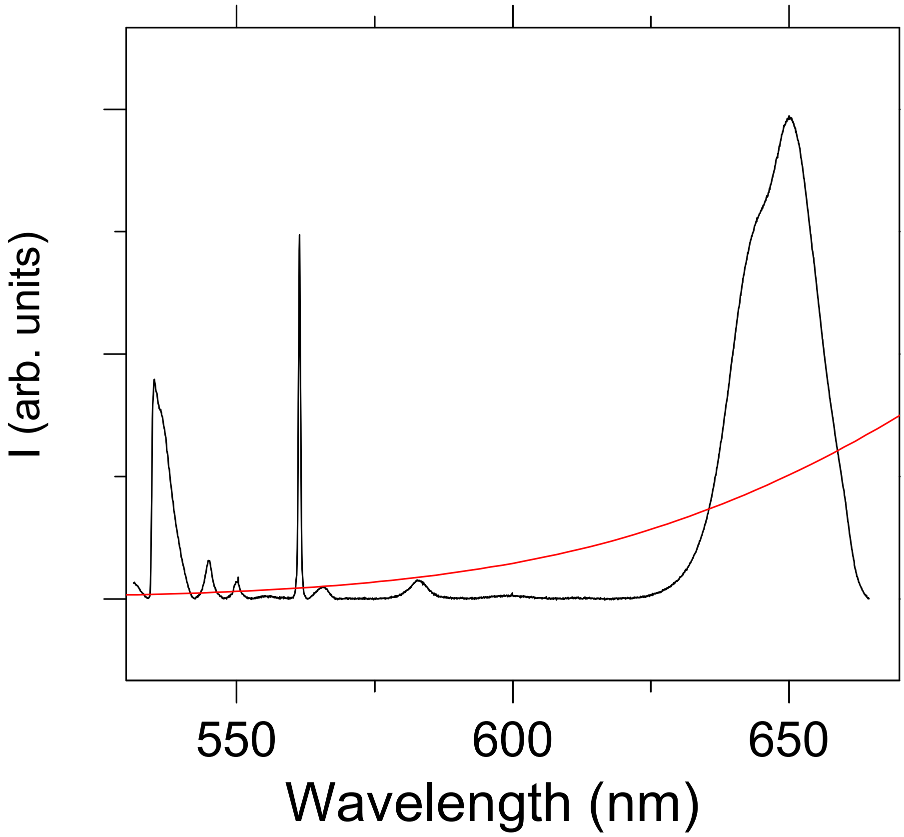

Figure 3. Absorption spectrum of $CuSO_4$ 0.4M solution, and a Raman spectrum of the same solution.

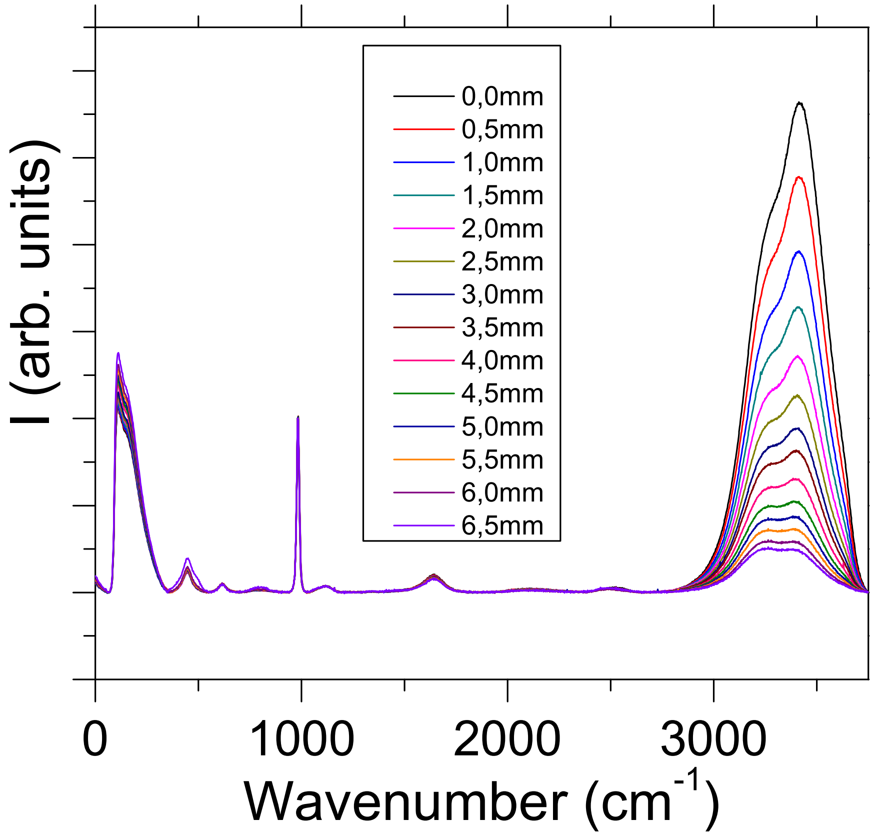

Figure 4. Raman spectrums of $CuSO_4$ 0.4M solution, taken with a depth difference of 0.5mm.

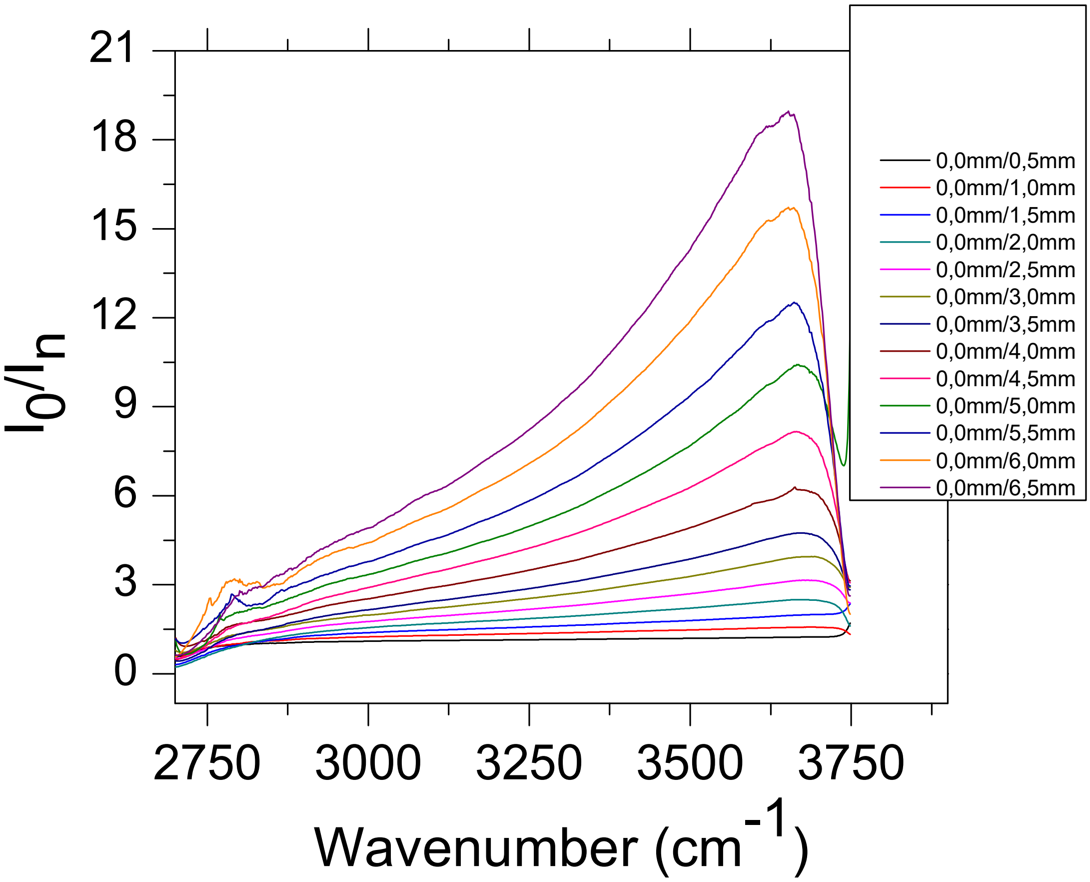

Figure 5. Absorption functions obtained from the relation I_0/I_n using the intensities of the Raman spectrums from Figure 4.

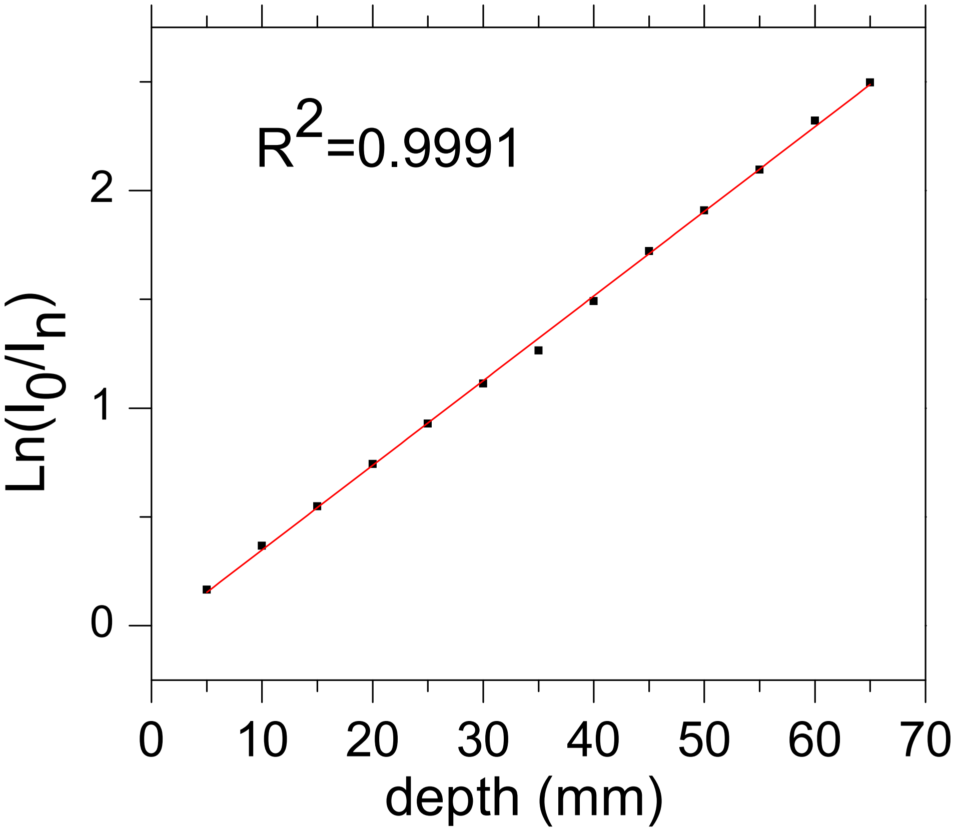

Figure 6. Calibration curve obtained using the Lambert-Beer law and the values of I_0/I_n from Figure 5 at 3410cm^{-1}.

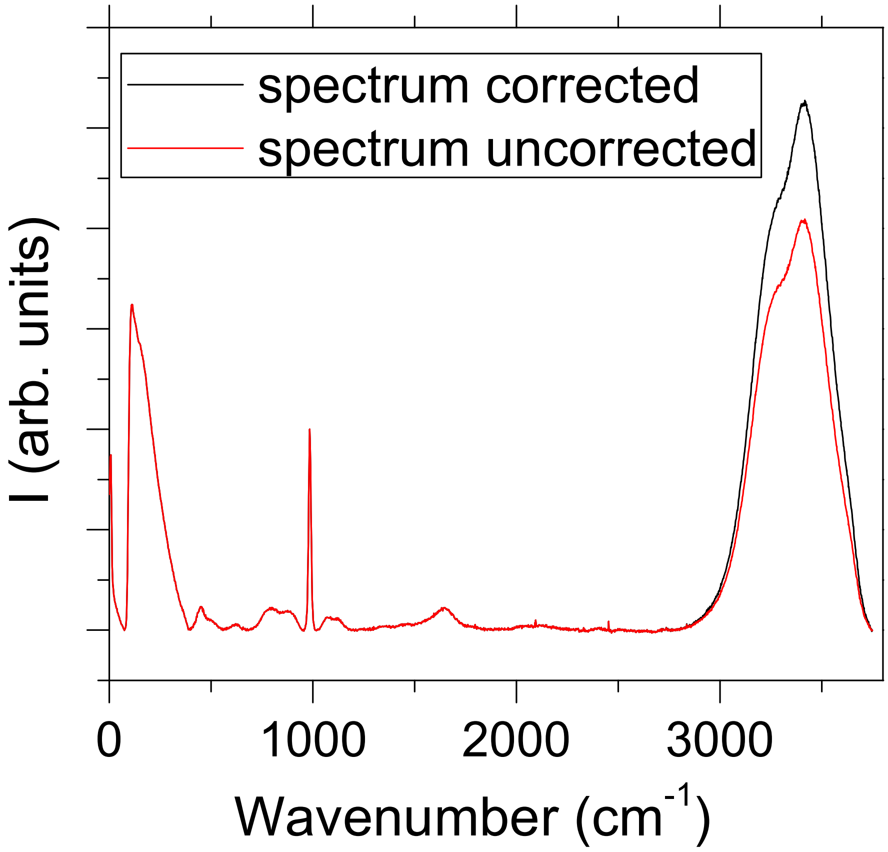

Figure 7. Raman spectrum taken at an unknown depth of a $CuSO_4$ 0.4M solution (red), and the correction of the spectrum using the method presented (Black).

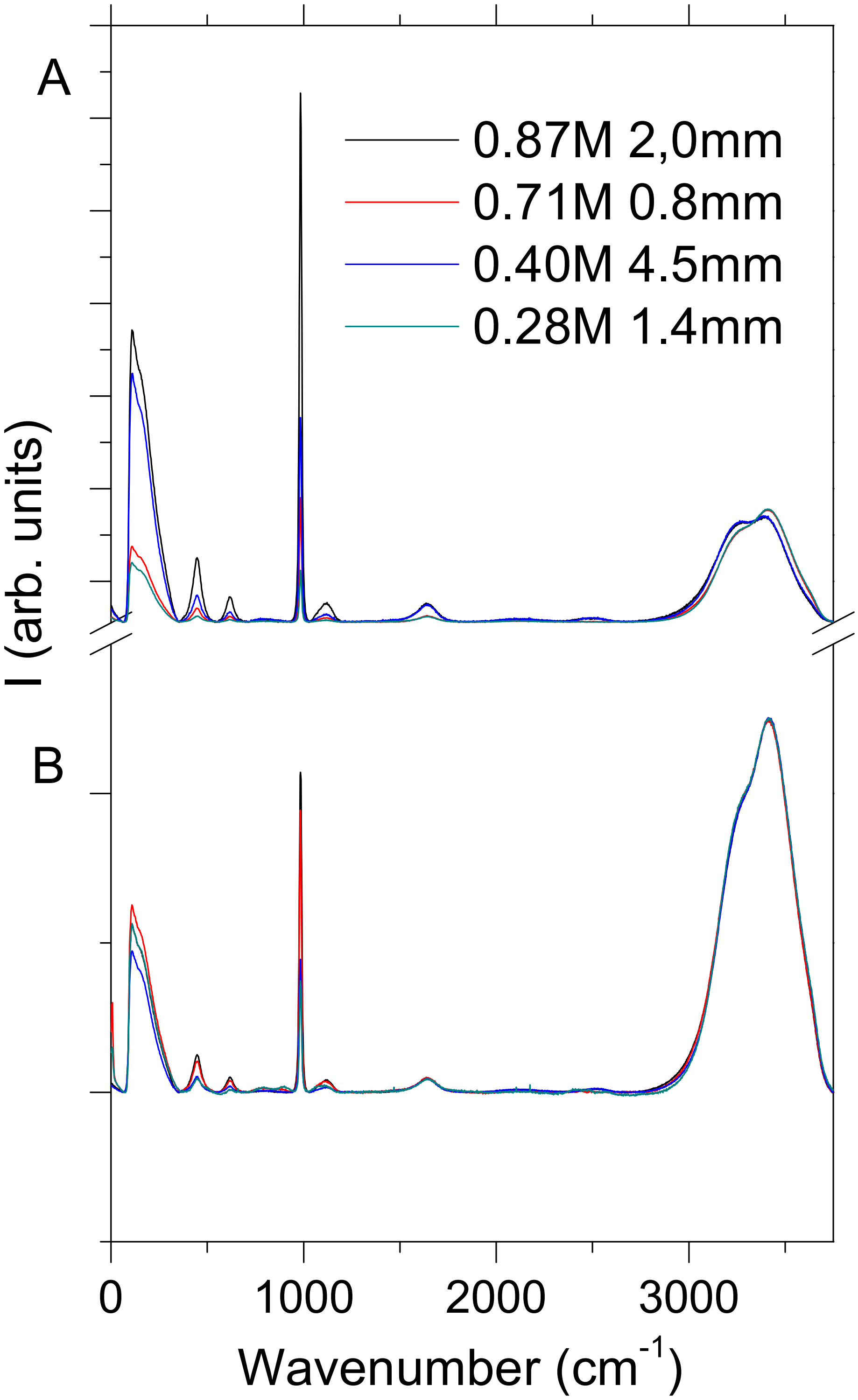

Figure 8. (A) Raman spectrums taken from different solutions at aleatories measures of depth and normalized with the area under the O-H stretch band. (B) The same spectrums corrected with the method presented on the work and ormalized with the new area under the O-H stretch band.

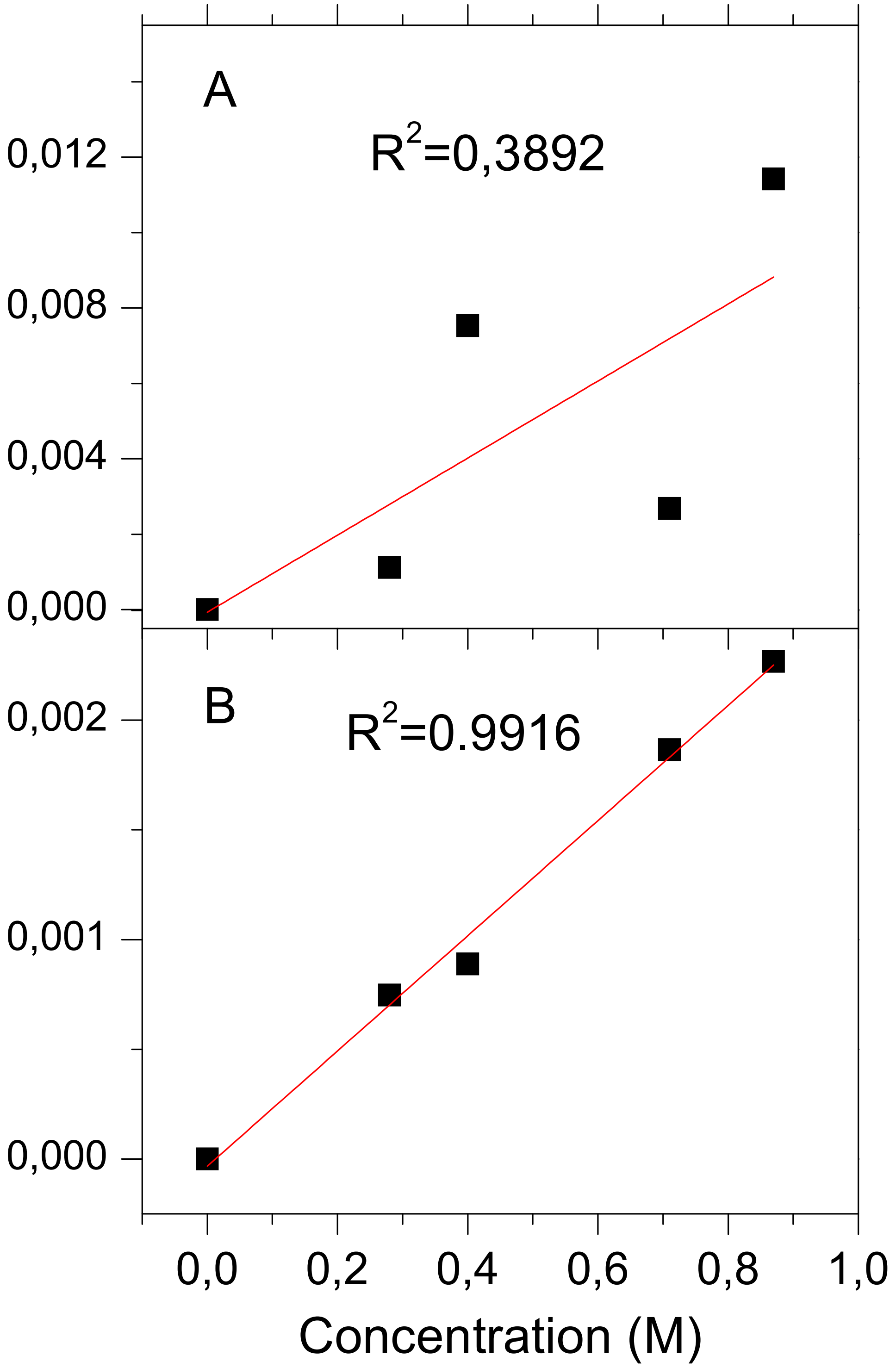

Figure 9. Calibration curves obtained using the intensity of the maximum of the S-O stretch (984.29cm^{-1}), from Figure 8.