Resonance surface enhanced Raman optical activity of myoglobin as a result of optimized resonance surface enhanced Raman scattering conditions

2007 J. Phys.: Condens. Matter 19 285205

(http://iopscience.iop.org/0953-8984/19/28/285205)

IOP PUBLISHING JOURNAL OF PHYSICS: CONDENSED MATTER

J. Phys.: Condens. Matter **19** (2007) 285205 (8pp) doi:10.1088/0953-8984/19/28/285205

Resonance surface enhanced Raman optical activity of myoglobin as a result of optimized resonance surface enhanced Raman scattering conditions

Salim Abdali[1], **Christian Johannessen, Jesper Nygaard and Thomas Nørbygaard**

Quantum Protein Centre, QuP, Department of Physics, Technical University of Denmark, DK-2800 Kongens Lyngby, Denmark

E-mail: abdali@fysik.dtu.dk

Received 29 September 2006, in final form 4 April 2007
Published 25 June 2007
Online at stacks.iop.org/JPhysCM/19/285205

Abstract

Using surface enhanced ROA (SEROA), novel results are achieved by combining Raman optical activity (ROA) and resonance surface enhanced Raman scattering (SERRS), applied on myoglobin. The novelty of this work is in reporting for the first time on chiral results of a study performed on a protein at single molecule level. This work, using silver nanoparticles and a laser excitation of 532 nm, only became feasible when the concentrations of nanoparticles, aggregation agent NaCl and the studied molecule were optimized in a series of systematic optimization steps. The spectral analysis has shown that the SERS effect behaves accordingly, depending on the concentration ratio of each component, i.e., myoglobin, Ag colloids and NaCl. Consequently, it is shown here that the SERS intensity has its maximum at a certain concentration of these components, whereas below or above this value the intensity decreases. The optimization results can be considered as a completion of the hitherto known phenomenon 'dilution effect', which only takes account of higher concentrations. Furthermore, the optimization of the parameters seems to be necessary for a successful SEROA measurement, which enables chiral study of a protein at the single molecule level, in which the concentration and acquisition time are no longer an impediment.

1. Introduction

The surface enhanced Raman scattering (SERS) effect [1–3] is a growingly applied tool, and today it covers main areas in science such as physics, chemistry and biology, and its applications can be found for bigger systems such as living cells, drugs complexes and proteins [4–8] as well as for smaller systems such as amino acids and nucleic acids [9, 10].

[1] Author to whom any correspondence should be addressed.

SERS can provide typical enhancements of 10^4–10^6 times that provided by conventional Raman spectroscopy (RS), and so far an enhancement of up to 10^{14} has been reported [11]. This means that the high concentrations required in RS (normally 10^{-2} M) may in principle be reduced to 10^{-16} M, and hence detection on the single molecule level can be achieved [12, 13]. Moreover, although the advanced optical technology employed in the state-of-the-art Raman apparatus has brought the time detection in RS down to milliseconds (Hug, ICORS2002, and Nafie, ICORS2004), the concentration is still in a much higher range than it is needed in many applications. Today, SERS as a spectroscopy tool is widely used for molecular detection and structural studies due to its ultra-high sensitivity and surface selectivity ([14, 15] and references therein). However, since the discovery of the SERS effect, it has not been considered as a trivial task, simply because in this process there are different variables involved, and their interplay and interaction dynamics add more complexity to the process.

In general, the enhanced effect has been described due to chemical as well as physical electromagnetic mechanisms [16–18]. Because this effect is dependent on the rough surface of nanoparticles, the size of the nanoparticle has been given high consideration to understand this process. Different results have been reported, showing the dependence of SERS on the concentration of the colloids [19–23]. However, we have found here that the concentrations of the other competing components in the SERS effect, i.e. the aggregating salt and the studied molecule, should also be considered. In the following we will show how important these components are, and consequently that individual studies taking only one or another component into account may lead to faulty conclusions.

Most importantly, by applying the optimized conditions we were able for the first time to conduct resonance chiral experiment on a protein, namely myoglobin (Mb), in which silver colloids were employed, hence enhancement in the chiral signals was obtained. We consider the present work as a novel result, as it becomes obvious that only by carrying out a series of optimization (of the sample components) measurements could an ROA spectrum of Mb be obtained in a considerably shorter time than usually known for conventional ROA. These results will open the way to further understanding of proteins, their chirality and folding on the single molecule level. It is here worth mentioning that in spite of the power of ROA as a chiral tool [24, 25], it requires higher sample concentration and moreover, in contrast to RS, longer acquisition time. The limitation of ROA applications and the reluctance of its spread can be ascribed these factors. Nevertheless, only by combining the surface enhanced effect and ROA (SEROA) could high concentration and long acquisition time be overcome.

A single SEROA study of a chiral molecule has so far been reported [26, 27]. However, this tool was employed to study a chiral pentapeptide, enkephalin, which is the natural painkiller in many living species. In the following, we report on observation of SEROA from a protein (Mb), obtained by a series of optimizations of the concentration of the measured protein, Ag colloids and NaCl used as an aggregating agent. The optimization seemed to be necessary for obtaining a successful SEROA. Based on this optimization and the results achieved in this study, another attempt has been made to measure another molecule (cytochrome c [33]), which showed that SEROA could be achieved when the same optimized conditions were applied. In this work we have also shown that the enhancement effect was not due to resonance, but also due to the existence of the molecule in the vicinity of metal nanoparticles.

2. Experiment and materials

2.1. Nanoparticles

The nanoparticles, silver colloids, were prepared according to the Lee and Meisel [28] procedure. Here, 90 mg $AgNO_3$ (Merck, extra pure) was dissolved in 500 ml milliQ H_2O

and brought to near boiling in a closed system. 10 ml of 1% sodium citrate (Aldritch, 99%) solution was added and the mixture was boiled under reflux for 90 min. The resulting solution was opaque and greyish with a silver colloid concentration of 114 mg l^{-1}. The size of the colloids was measured by UV–vis spectrometry using a Cary-3 spectrometer, which showed a peak around 406 nm, corresponding to an average size of 50 nm in diameter, and this was also confirmed by a scanning electron microscopy measurement.

2.2. Sample preparation

Myoglobin is a 153 amino acid protein, corresponding to a MW of ca 17 000 Da. Structurally well defined as an all α-helical protein with a haem prosthetic group, Mb functions as an oxygen-storage molecule in muscle, and is hence abundant in all mammals. Oxygen is stored by coordination to the central Fe ion of the haem group. In this work, freeze dried horse Mb (95% purity) was purchased from MP Biomedicals and used without further purification. The protein was dissolved in milliQ H_2O at 100 μm stock concentration, and subsequently diluted as needed (see section 3, below).

2.3. Spectral recording

Raman spectra were recorded on a Chiral*R*AMAN instrument (BioTools Inc., USA), which simultaneously provides both Raman and ROA spectra. The instrument utilizes a 532 nm laser source (Excel, LaserQuantum, UK) and a back-thinned CCD detector, which is optimized for recording in the spectral range 100–2400 cm^{-1}. Spectral resolution, limited by the width of the individual optical fibres at the entrance of the spectrograph, is approximately 7 cm^{-1}.

The spectra were verified by measuring the colloid solution, the colloids and the salt and Mb in the mixture of colloids and salt. The first two spectra showed no Raman spectra, nor ROA.

3. Results and discussion

3.1. Concentration optimizing conditions

A series of SERS measurements on Mb has been carried out in order to achieve the best-optimized condition, which yields higher peak intensity at the lowest concentration. In this several factors have been taken into account, especially the components that have biggest interplay in this process. These components include the concentration of the protein, Mb, the colloids, Ag, and the aggregation agent, NaCl.

In the following, measurements of different concentrations of Mb in fixed Ag and NaCl concentrations are first shown, followed by different Ag concentrations and then different NaCl concentrations. The recording parameters were kept the same in all these measurements, i.e. sample volume (200 μl), and all spectra were recorded using 32 scans, each with illumination time of 3 s.

Figure 1(a) shows resonance SERS intensity as a function of Mb concentration in the range 10^{-6}–5×10^{-8} M, whilst figure 1(b) shows how the intensities of two peaks appear in the resonance SERS spectra of Mb as a function of Mb concentration in the same range as in figure 1(a). The two peaks assigned to symmetric (1374 cm^{-1}) and anti-symmetric (1169 cm^{-1}) ν (pyr half-ring) are plotted in solid and dashed lines, respectively, as a function of the corresponding concentration in figure 1(b). These peaks have been chosen due to the fact that they are found to be independent of the noise ratio, which occurs mainly due to fluorescence in resonance SERS spectra. It is worth mentioning that similar measurements

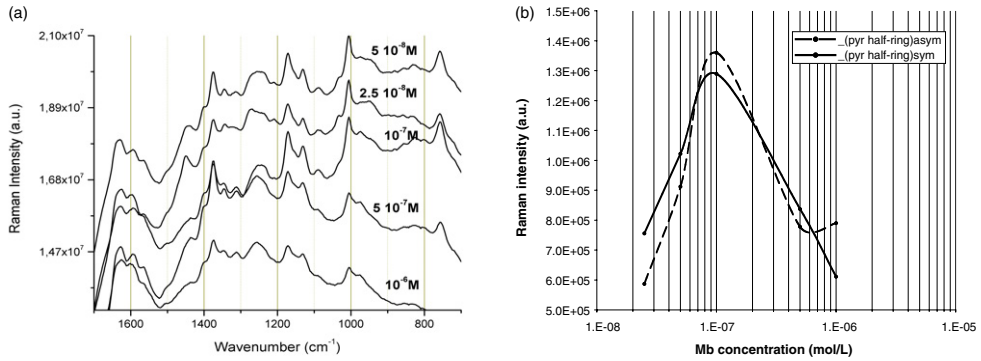

Figure 1. Resonance SERS spectra of Mb as a function of concentration (a) in the range 10^{-6} M to 5×10^{-8} M; (b) the intensity as a function of concentration of symmetric (solid line) and anti-symmetric (dashed line) ν (pyr half-ring) at 1374 cm^{-1} and 1169 cm^{-1}, respectively.

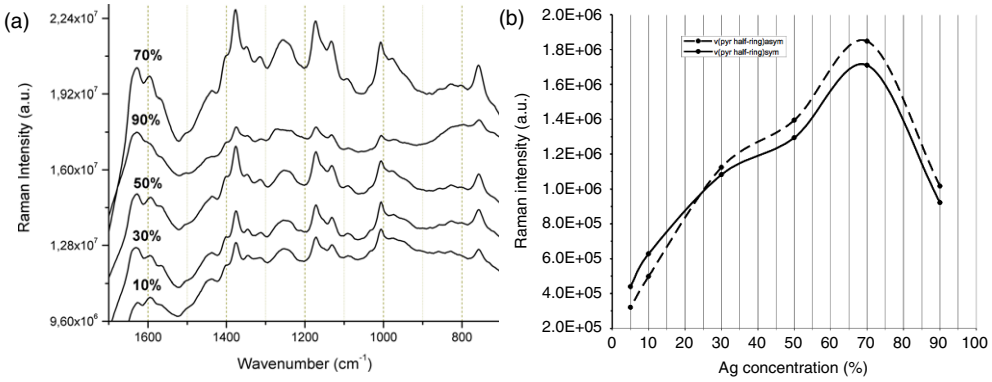

Figure 2. Resonance SERS spectra of Mb as a function of Ag colloid concentration (a) in the range 10–90%; (b) the intensity as a function of Ag colloid concentration of symmetric (solid line) and anti-symmetric (dashed line) ν (pyr half-ring), at 1374 cm^{-1} and 1169 cm^{-1}, respectively.

were carried out below and above the presented concentration range. However, outside this range, the intensity was not resolved properly. The results in figure 1 were achieved by varying Mb concentration whilst having both Ag colloids and NaCl concentrations fixed with respect to the total concentration of the measured sample. The results, plotted in figure 1(b), show clear dependence of the intensity on Mb concentration, in which it is proportional at lower concentrations below the maximum value, at 10^{-7} M, and it is inversely proportional when this value is exceeded.

Resonance SERS intensity of the above mentioned peaks were also measured as a function of Ag colloid concentration in the range 10–90% of the measured sample. The resonance SERS spectra of these measurements can be seen in figure 2(a), and the intensity of the symmetric (1374 cm^{-1}) and anti-symmetric (1169 cm^{-1}) ν (pyr half-ring), plotted in solid and dashed line, respectively, as a function of Ag concentration, can be seen in figure 2(b). Considering these measurements, it is obvious that a concentration of 70% results in the highest intensity for both peaks.

The concentration of NaCl, used as an aggregating agent for these colloids, is from a previous work [29] known to affect the enhancement process, in that it plays a role in

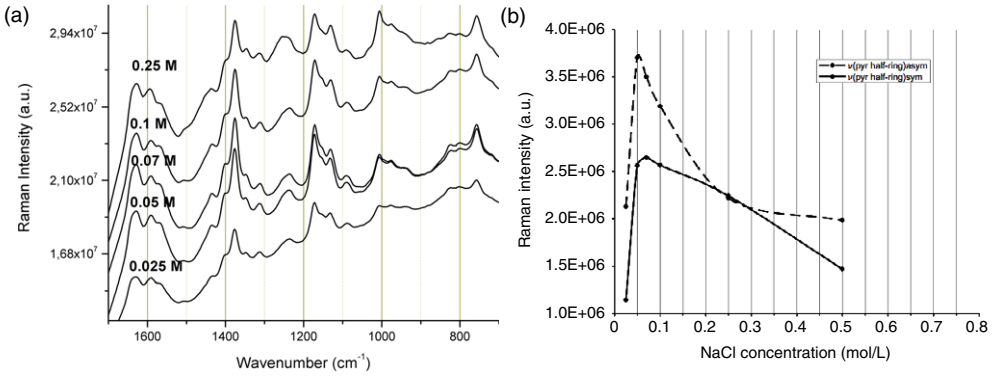

Figure 3. Resonance SERS spectra of Mb as a function of aggregation agent, NaCl, concentration (a) in the range 0.025–0.25 M; (b) the intensity as a function of NaCl concentration of symmetric (solid line) and anti-symmetric (dashed line) ν (pyr half-ring), at 1374 cm^{-1} and 1169 cm^{-1}, respectively.

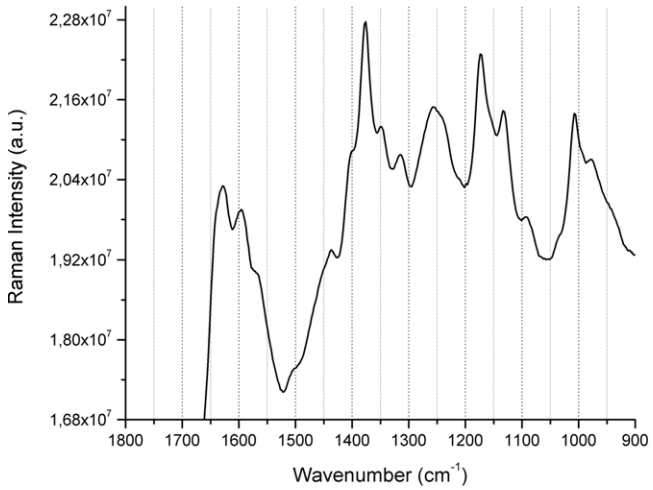

Figure 4. Resonance SERS spectrum of Mb, in which the optimized conditions were employed.

aggregation of the colloids, and hence a better enhancement can be achieved. Having fixed Mb and Ag concentrations in the sample, new measurements were carried out in order to obtain the best concentration of NaCl to be used. Figure 3 shows clearly that a concentration of 0.05–0.07 should be used in order to achieve the best intensity of Mb as a function of concentration.

Accordingly, the optimization measurements conclude that using Mb of concentration of 10^{-7} M in a solution containing 70% Ag colloids and 0.05 M NaCl provides the best resonance SERS spectrum. A spectrum in which these concentrations were employed, can be seen in figure 4.

Here, it is worth mentioning that, whilst the number of molecules per unit volume counts when measuring Raman spectra, the number of molecules currently adsorbed at SERS active sites will influence the detected intensity in SERS spectra. This explains the intensity behaviour seen in figure 1, in which initially as the sample concentration increases the number of SERS active sites will also increase, and consequently higher SERS intensity will be obtained. This

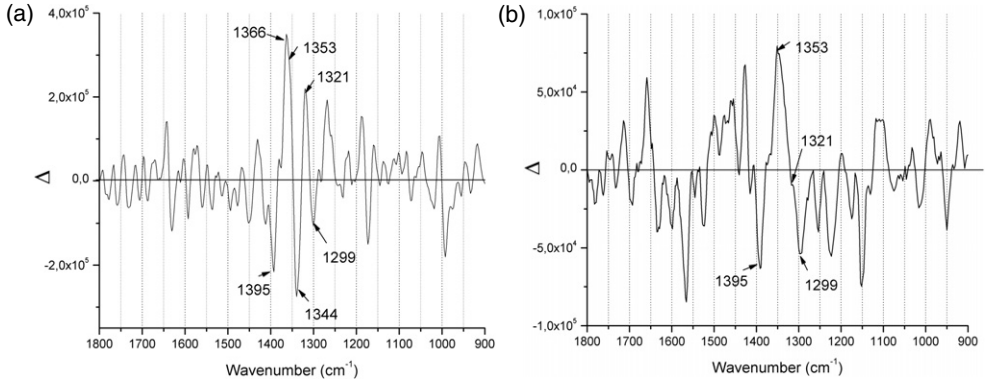

Figure 5. Resonance ROA spectrum measured for 32 h (a), and resonance SEROA spectrum measured for 5 min (b). The chiral peaks show a clear resemblance, especially in the frequency range 1400–1300 cm^{-1}. This region also exhibits the biggest enhancement in resonance SERS measurements, seen in figure 4.

will continue until a maximum limit has been reached, beyond which any increase in the sample concentration will yield a decrease in SERS intensity due to an increase in the background or/and an increased fluorescence, which results from the sample molecules aggregating, hence an increase of opacity, especially in the case of a coloured sample, like Mb.

3.2. SEROA measurements

In this section we will show that, similar to the resonance SERS, it is possible to achieve a corresponding chiral enhancement (SEROA) for Mb, using the optimized conditions obtained in section 3.1.

Due to the inherent tendency of artifact occurrence in an ROA spectrum recorded in a short time (<1 h), and in order to ensure the stability of the colloidal aggregates, a measurement was carried out for 60 min. However, the chiral peaks in the spectrum were pronounced already after few minutes, but they were not stable during the detection, hence we limited our observation to 5 min, as shown in figure 5(b). Nevertheless, the Raman signal was simultaneously monitored for erratic hot-spots, and found to be stable during the experiment. In order to verify the authenticity of the surface enhanced effect, a resonance ROA spectrum of Mb of 100 μM concentration was also recorded, as shown in figure 5(a). It should be mentioned that the 32 h acquisition was necessary in order to achieve sufficient signal to noise ratio.

Comparing the spectra of resonance ROA in figure 5(a) and resonance SEROA in figure 5(b), it is noticeable that the chiral signals of Mb, marked with their corresponding frequencies, appear at the same frequencies in both spectra. In particular, the region 1400–1300 cm^{-1}, which also exhibits the largest signal enhancement in the resonance SERS spectrum, seen in figure 4, has a considerable signature overlap around 1395, 1353, 1321 and 1299 cm^{-1}. The peak around 1353 cm^{-1} in figure 5(b) appears to have bigger FWHM than the similar peak seen in the ROA spectrum, shown in figure 5(a). This may explain the absence of the peaks around 1366 and 1344 cm^{-1} seen in figure 5(a). All peaks in this region have previously been assigned to vibrations of the haem group in the protein, and have been shown to be enhanced by the resonance Raman effect using the appropriate laser excitation, i.e. 532 nm [29–32].

A conventional ROA spectrum of Mb has peaks that cannot be assigned to protein backbone vibrations, as their contribution to the spectrum would not be seen due to the lower

concentration, i.e., 100 μM. Therefore, the peaks found in the ROA spectrum in figure 5(a) must be due to the resonance enhancement of the porphyrin vibrations. Adding the colloids and the aggregation agent, NaCl, the ROA is further enhanced and a combination of resonance and surface enhancement of the chiral signals now takes place. We therefore believe that the results presented in figure 5 are genuine ROA signals, strongly enhanced by surface enhancement combined with resonance enhancement, yielding to resonance SEROA.

The observation of a SEROA from a protein can find important applications, and will introduce a new analytical tool of biological relevance, by considerably reducing the concentration needed for conventional ROA measurements. Moreover, reducing the measuring time immensely, e.g., to a few minutes of acquisition in SEROA compared to 32 h acquisition of ROA, will hopefully result in new applications of the method in research and industry.

4. Conclusion

Resonance surface enhanced Raman scattering (SERRS) and resonance surface enhanced Raman optical activity (SERROA) using Ag colloids and NaCl were used to study Mb. Resonance SERS measurements were carried out in a series of optimization conditions of the concentration of Mb, Ag colloids and the aggregation agent NaCl. The results showed that using Mb concentration of 10^{-7} M in a solution of 70% volume Ag colloids and NaCl of 0.05–0.07 M concentration provides the best spectrum recorded for this molecule. These conditions were further used for ROA measurements and showed a corresponding enhancement for only a few minutes of recording. These results are considered as novel results, and may open new applications, especially in medicine and biology due to the short time (few minutes) detection and the low concentration of the sample ($<10^{-7}$ M).

Acknowledgments

Professor L D Barron and Dr L Hecht are acknowledged for fruitful discussion, and Kristian E Krantz for his help in data collection. This work was supported by the Danish Fundamental Research Foundation (2001–2009).

References

[1] Fleishmann M, Hendra P J and McAuillan A J 1974 *Chem. Phys. Lett.* **24** 163
[2] Jeanmaire D L and Van Duyne R P 1977 *J. Electroanal. Chem.* **84** 1
[3] Albrecht M G and Greighton J A 1977 *J. Am. Chem. Soc.* **99** 5215
[4] Kim S K, Kim M S and Suth S W 1987 *J. Raman Spectrosc.* **18** 171
[5] Dou X, Jung Y M, Cao Z-Q and Ozaki Y 1999 *Appl. Spectrosc.* **53** 1440
[6] Watanabe T and Maeda H 1989 *J. Phys. Chem.* **93** 3258
[7] Suh J S and Moskovits M 1986 *J. Am. Chem. Soc.* **108** 4711
[8] Chumanov G D, Efromov R G and Nabiev I R 1991 *J. Raman Spectrosc.* **21** 43
[9] Tessier P M, Velev O D, Kalambur A T, Rabolt J F, Lenhoff A M and Kaler E W 2000 *J. Am. Chem. Soc.* **122** 9554
[10] Olson L G, Lo Y-S, Beebe T P Jr and Harris J M 2001 *Anal. Chem.* **73** 4268
[11] Kneipp K, Kneipp H, Itzkan I, Dasari P R and Feld M S 1999 *Chem. Rev.* **99** 2957
[12] Kneipp K 1988 *Exp. Tech. Phys.* **36** 161
[13] Kneipp K, Wang Y, Dasari P R and Feld M S 1995 *Appl. Spectrosc.* **49** 78
[14] Brolo A G, Irish D E and Smith B D 1997 *J. Mol. Struct.* **405** 29
[15] Campion A and Khambhampati P 1998 *Chem. Soc. Rev.* **27** 241
[16] Otto A 1984 *Light Scattering in Solid* ed M Cardona and G Guentherodt (Berlin: Springer)
[17] Moskovits M 1985 *Rev. Mod. Phys.* **57** 783

[18] Garcia-Vidal F J and Pendry J B 1996 *Phys. Rev. Lett.* **77** 1163
[19] Link S, Wang Z L and El-Sayed M A 1999 *J. Phys. Chem.* B **103** 3529
[20] Xue G and Shi G 1994 *Instrum. Sci. Technol.* **22** 179
[21] Xue G, Lu Y and Gao J 1994 *Appl. Surface Sci.* **78** 11
[22] Glaspell G P, Zuo C and Jagodzinski P W 2005 *J. Cluster Sci.* **16** 39
[23] Clark R J H and Dines T J 1986 *Angew. Chem. Int. Edn Engl.* **25** 131
[24] Barron L D, Hecht L, Blanch E W and Bell A F 2000 *Prog. Biophys. Mol. Biol.* **73** 1
[25] Barron L D, Hecht L, McColl I H and Blanch E W 2004 *Mol. Phys.* **102** 731
[26] Abdali S 2004 *Proc. ICORS (Brisbane, Australia)*
[27] Abdali S 2007 *J. Raman Spectrosc.* **37** 1341 (online since May 2006)
[28] Lee P C and Meisel D 1982 *J. Phys. Chem.* **86** 3391
[29] Nygaard J 2006 Determination of myoglobin by Raman and SERS spectroscopy *MSc Thesis* Quantum Protein Centre, QuP, Department of Physics, Technical University of Denmark
[30] Bizzari A R and Cannistrato S 2002 *Appl. Spectrosc.* **56** 1531
[31] Hu S, Smith K M and Spiro T G 1996 *J. Am. Chem. Soc.* **118** 12638
[32] Schweitzer-Stenner R and Dreybrodt W 1992 *J. Raman Spectrosc.* **23** 539
[33] Johannesse C, White P and Abdali S 2007 *J. Phys. Chem.* B at press