CARS and P-CARS Measurements of Ion Concentration in Electrolyte Solution: Influence of Non–resonant Background Noise on Non–intrusive Detection

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Keywords: Coherent anti-Stokes Raman Scattering, Electrolyte solution, Mixing flow field, Polarization–CARS, Non–resonant background noise

ABSTRACT
Non–intrusive concentration sensing systems were developed to investigate the ion motion in micro-scale mixing flow fields of electrolyte solutions using CARS (coherent anti–Stokes Raman Scattering) and P-CARS (Polarization–CARS). First, a CARS system was developed. To confirm the measurement ability of the CARS system, a calibration experiment was conducted using Na₂SO₄ and NH₄Cl solutions with various concentrations. In the calibration experiment, the relationships between the solution concentrations and CARS light intensity ratio were obtained. Moreover, the concentration profiles of Na₂SO₄ and NH₄Cl in a solution mixing field were obtained using the obtained relationships. Although the concentration gradient was observed in the junction area for each solution component, the concentration value measured near the boundary of two solutions were higher than those predicted by a diffusion theory because of the non–resonant background noise generated from every molecules. Then, for the noise elimination, a P-CARS system was developed. This system separates the noise and CARS light by polarization directions. And the noise was eliminated by polarization plate. To confirm the noise eliminating effect provided by P-CARS, a calibration experiment using mixed solutions and concentration measurements in a solution mixing field were conducted. In the calibration experiment, the relationships between the Na₂SO₄ concentration in Na₂SO₄/NH₄Cl mixed solutions and CARS light intensity ratio were obtained. These results show that NH₄Cl does not influence the calibration curves obtained by using the P-CARS system, in contrast to the CARS system. Moreover, a concentration profile of Na₂SO₄ in a mixing field was obtained. The non–resonant background noise cancellation effect of P-CARS was confirmed by comparing the results obtained by using the CARS system and the P-CARS system.

1. Introduction
Recent years, microfluidic devices, such as µ–TAS (micro-total analysis system) or lab–on–a–chips, are developed for biological and chemical reactions (cf. Arora A. et al. (2010) and Flovio C. C. et al. (2015)). In these devices, various chemical processes, for example, mixing, reaction or
separating, are performed. Such chemical operations in micro scale have advantages in amount of samples and times for process compare to those in macro scale. To enhance the efficiency of these chemical processes, comprehending the condition in the device are required. As a general micro-scale sensing technique, Laser Induced Fluorescence (LIF) is well known (cf. Ichyanagi et al. (2007)). This technique is often used for measurement in flow field, because it is helpful for obtaining two dimensional scalar quantity with high sensitivity. However, this technique requires a fluorescent dye to be mixed in the sample, and there are some concerns about the measurement result using a fluorescent dye. Fluorescent dyes may adversely influence the sample and the fluorescence intensity would become unstable by photobleaching. Therefore, a fluorescence-free and non-intrusive measurement technique is required. Measurement techniques using Raman scattering fulfill this requirement (cf. Raman V. C. (1928)). Takahashi et al. developed an technique for ion concentration measurement in electrolyte solutions using spontaneous Raman scattering. However, the spontaneous Raman scattering is relatively weak. Therefore the spontaneous Raman scattering requires a long exposure time. In order to overcome this problem, this study focused on coherent anti-Stokes Raman scattering (CARS) whose intensity of scattering light is much higher than that of spontaneous Raman scattering. Moreover, CARS has non-intrusiveness and chemical specificity as is the case with the spontaneous Raman scattering. Maker et al. proposed the theory of CARS in 1964 and Duncan et al. observed the cells of onion peel using CARS in 1982. Additionally, Zumbusch et al. constructed the CARS microscopy system, which has three dimensional resolution, using high N.A. objective lens. This technique was applied to many researches in micro-scale (e.g. cellular imaging) (cf. Freudiger W.C. et al. (2008)). In recent years, quantitative techniques have been reported; the concentration measurement for glycine in hair, the observation for the acid-base reaction using concentration measurement in micro-reactor and the imaging of cellular lipid droplets based on the C=C and C-H stretching vibrations (cf. Lin Y. C. et al. (2009), Schafer D. et al. (2008) and Bonn M. et al. (2009)). However, the non-resonant background noise, which is characteristic to CARS measurement, have been concerned. This noise is scattered from the other samples, and the noise is scattered with the same wavelength as CARS light. It is impossible to measure the sample concentration accurately without eliminating the non-resonant background noise. Therefore, a new technique for eliminating the non-resonant background noise by considering polarization directions of the laser beams and scattering light, called polarization CARS, was proposed by Akhmanov et al..

The objective of this study is to develop a technique which realizes a non-intrusive, highly sensitive and quantitative ion concentration measurement. The goal of this study is to obtain the
ion concentration profiles in the electrolyte solutions using CARS and P-CARS systems developed in this study and to confirm the utility of the P-CARS system by a comparison of the results obtained by both the CARS and P-CARS systems.

2. Principle

2.1 Spontaneous Raman scattering

When an excitation beam is irradiated to a material, scattering lights, whose frequencies are different from that of the excitation beam, are generated by molecular vibrations. This phenomenon is called Raman scattering. Figure 1(a) shows a schematic of the Raman scattering. The scattering light whose frequency is lower than that of the excitation beam, is called Stokes Raman scattering, and the light whose frequency is higher than that of the excitation beam, is called anti–Stokes Raman scattering. Stokes and anti–Stokes Raman scatterings are generated through energy diagrams shown in figures 1(b) and 1(c), respectively. Raman shift $\omega$ is indicated in equation (1).

$$\frac{\nu_{\text{Raman}} - \nu_{\text{laser}}}{c} = \omega$$

Figure 1. (a) Schematic of Raman scattering and energy diagrams of (b) Stokes Raman scattering and (c) anti–Stokes Raman scattering.

2.2 Coherent anti-Stokes Raman scattering

Although non–intrusive concentration measurements can be realized by utilizing Raman scattering, Raman scattering light is difficult to obtain because of its weakness. In order to make use of the advantage and conquer the disadvantage of the measurement using Raman scattering, coherent anti–Stokes Raman scattering (CARS), which is the resonantly-enhanced form of anti–
Stokes Raman scattering, can be a powerful method. Figure 2(a) shows a schematic of the CARS light generation. A molecule is excited by two laser beams called pump and Stokes beams, whose frequencies are $\nu_{\text{pump}}$ and $\nu_{\text{Stokes}}$, respectively. The frequency difference between two beams must be correspond to the frequency of sample molecule. The intensity of the CARS light is expressed by equation (2);

$$I(\omega_{\text{CARS}}) = \varepsilon_0 \cdot N^2 \cdot \chi^{(3)}_{ijkl} \cdot E_{\text{pump}}^2 \cdot E_{\text{Stokes}}$$

(2)

where “$N$” is the number of molecules. $\varepsilon_0$, $\chi^{(3)}_{ijkl}$, $E(\nu_{\text{pump}})$ and $E(\nu_{\text{Stokes}})$ are permittivity of vacuum, the third order susceptibility, the electric fields of the pump beam and Stokes beam, respectively. $\chi^{(3)}_{ijkl}$ can be written as equation (3) by the non-resonant background susceptibility $\chi^{NR}_{ijkl}$ and Raman susceptibility $\chi^R_{ijkl}$.

$$\chi^{(3)}_{ijkl} = \chi^{NR}_{ijkl} + \chi^R_{ijkl}$$

(3)

The scattering light arising from $\chi^R_{ijkl}$ is called CARS light. The CARS light depends on the sample molecule and becomes zero in case the scattering light generated from excluded molecule. Therefore, the sample concentration is calculated from the intensity of CARS light, because the intensity of CARS light is proportional to the square value of the sample concentration. CARS light is generated through energy transition shown in figure 2(b). $\nu_{\text{pump}}$, $\nu_{\text{Stokes}}$ and $\nu_s$ are the frequencies of the pump beam, Stokes beam and sample molecules. $h$ is the Planck constant.

![Figure 2. (a) Schematic and (b) energy diagram of CARS.](image)

On the other hand, scattering light arising from $\chi^{NR}_{ijkl}$ are generated from not only sample molecules but also excluded materials. This light is called the non-resonant background noise. This noise is generated regardless of the species, and its frequency is the same as that of CARS light. Figure 3 shows a schematic of energy transition to generate the non–resonant background noise. This noise has no information about sample concentration and interferes the CARS measurement, therefore eliminating the non–resonant background noise is required.
2.3 Polarization-CARS

It is impossible to distinguish the non–resonant background noise and the CARS light when the polarization direction of the noise is the same as that of CARS light, because their frequencies are the same. Since both $\chi^R$ and $\chi^{NR}$ contain the information of the polarization directions, polarization-CARS (P-CARS), which separates the noise and CARS light with polarization directions, was proposed by Akhmanov et al.. When pump and Stokes beams are irradiated to the material, the x and y direction polarization vectors of the CARS light, which are shown in figure 4, are expressed as equation (4)(5), respectively.

$$P_x = 3\cos\phi(\chi^{NR}_{1111} + \chi^R_{1111})E_{pump}^2E_{Stokes}$$

$$P_y = 3\sin\phi(\chi^{NR}_{1221} + \chi^R_{1221})E_{pump}^2E_{Stokes}$$

$\chi^{NR}$ must be real quantity, therefore $\alpha$ is expressed as:

$$\alpha = \cos^{-1}\left(\frac{\chi^{NR}_{1221}}{\chi^{NR}_{1111}}\right)\tan\phi$$

And $\alpha$ is expressed as equation (7) using $\phi$ by applying the Kleinman symmetry to $(\chi^{NR}_{1221} / \chi^{NR}_{1111})$.

$$3\cos\alpha = \tan\phi$$

From the above, $P_x$ and $P_y$ can be expressed as equation (8)(9).

$$P_x = \left(3\chi^{NR}_{1111} \cos\phi / \cos\alpha + 3\chi^R_{1111} \cos\phi \cos\alpha \times (1 + \chi^R_{1221} \tan\phi \tan\alpha / \chi^{NR}_{1111})\right)E_{pump}^2E_{Stokes}$$

$$P_y = \left(-3\chi^{NR}_{1111} \cos\phi \sin\alpha \times (1 - \chi^R_{1221} \tan\phi \tan\alpha / \chi^{NR}_{1111})\right)E_{pump}^2E_{Stokes}$$

Moreover, $\alpha$ and $\phi$ must be 30° and 60° because of the equation (7). Therefore, only CARS light but the non-resonant background noise can be obtained by the component of $P_y$ at , and the
polarization directions of the CARS light and the noise are changeable by adjusting polarization directions of the excitation beams. (cf. Ouder J. L. et al. (1979) and Brakel B. et al. (1981))

3. Experiments and Results

3.1 Selection of the wavelengths of the excitation lights

Prior to the measurement using CARS, Spontaneous Raman spectra of Na₂SO₄ and NH₄Cl solutions were obtained. Figures 5(a) and 5(b) show the spectra of spontaneously emitted Raman scattering lights. The Na₂SO₄ and NH₄Cl have their specific peak at 982 cm⁻¹ and 3400 cm⁻¹, respectively. It is confirmed that the values of Na₂SO₄ and NH₄Cl concentration are obtained by detecting the intensity of the scattering light at their specific Raman shift. In the following experiments, the wavelength difference of the excitation beams were adjusted to the spaciRaman shift obtained in order to fulfill the condition of CARS generation: |ν_pump - ν_Stokes| = ν_R. Table 1 shows the wavelengths of each beams and resulting CARS light calculated based on the specific Raman shift.

![Spontaneous Raman scattered lights](image)

Figure 5. Spontaneous Raman emitted from (a) Na₂SO₄ and (b) NH₄Cl.

Table 1. Wavelength of pump and Stokes beams and resulting CARS light

<table>
<thead>
<tr>
<th></th>
<th>Pump light</th>
<th>Stokes light</th>
<th>CARS light</th>
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<tbody>
<tr>
<td>Na₂SO₄</td>
<td>737.5 nm</td>
<td>795 nm</td>
<td>688 nm</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>641 nm</td>
<td>820 nm</td>
<td>526 nm</td>
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</table>

3.2 CARS system

Figure 6(a) shows a concentrational CARS system (i.e., not P-CARS) developed in this study. A Ti:Sapphire pulsed laser (Coherent, Inc., Chameleon Ultra II, oscillation wavelength; 680-1080 nm) was employed as a source of excitation beams. The laser pulse repetition rate was 80 MHz and the pulse width was 140 fs. The laser beam was divided into two beams. One beam was
used as the Stokes beam after the wavelength conversion by an optical parametric oscillator (Coherent, Inc., Chameleon Compact OPO). And the other was used as the pump beam.

This CARS system has two microscopes equipped with a pair of objective lenses (Nikon Corp., N.A. = 0.75; WD = 1.0) (Nikon Corp., N.A. = 0.5; WD = 1.0), a filter block, a spectrometer (SOLAR TII Ltd., SL100M, 400 groove/mm, wavelength resolution < 0.57 nm) and an EM-CCD camera (Hamamatsu Photonics K. K., C9100-13, 512 × 512 pixels, 16 × 16 µm²/pixel, 16 bits). In order to generate the CARS light, pump and Stokes beams were overlapped coaxially by a dichroic mirror, and the pulse timings of these beams were synchronized by changing the light path length of the pump beam with a micromotion stage. These beams were focused by objective lenses on the stages of the inverted microscopes. One microscope detects CARS light generated from the sample. Figure 6(b) shows the schematic of CARS generation area. A region where the light intensity is higher than half of the peak intensity between a pair of objective lenses was defined as the area where CARS was generated. The size of the CARS generation area was calculated to be 22.4 µm in the direction of optical axis and 6.4 µm on the focal plane.

Figure 7(a) shows a picture of CARS light, which passed through the spectrometer and detected by the EM-CCD camera. Figure 7(b) shows the CARS spectrum at the red line in figure 7(a). Average of the light intensity within the full width half maximum of the spectrum, was defined as a CARS light intensity. The other microscope detects the reference value for the calculation of the CARS light intensity ratio, which reduces the influence of the intensity fluctuation of the excitation beams.

Figure 6. (a) Schematic of CARS system and (b) CARS generation area.
3.3 Experiments using CARS system and Results

3.3.1 Calibration experiment

For the first step to establish the concentration measurement technique using CARS, a calibration experiment was conducted. In this experiment, relationships between CARS light intensity ratio and the solution concentration were obtained. This experiment was conducted using an I-shaped channel whose detail is shown in Figure 8. The measurement point was midpoint of the channel. Solutions used for this experiment is Na$_2$SO$_4$ (0.2 0.4 0.6 0.8 1.0 mol/L) and NH$_4$Cl (1.0 2.0 3.0 4.0 mol/L). 1 mol/L of Na$_2$SO$_4$ and NH$_4$Cl solutions were used for the reference in the Na$_2$SO$_4$ and NH$_4$Cl concentration measurement, respectively.

![Figure 8. (a) Top and (b) cross sectional views of I-shaped channel.](image)

Figures 9(a) and 9(b) show the results of the calibration experiment. Calibration curves were obtained by the quadratic function approximation. It is confirmed that the relationships between the CARS light intensity ratio and both Na$_2$SO$_4$ and NH$_4$Cl concentration are expressed by quadratic functions.
3.3.2 Concentration measurements in mixing field

The solution concentration profile in a mixing flow field in a Y–shaped channel was obtained. The mixing field was at the junction area of the channel. Figures 10(a) and 10(b) show details of the Y-shaped channel. The inlets of the channel were connected to a syringe pump (KD Science Inc., LEGATO 210), and 1.0 mol/L of Na$_2$SO$_4$ solution and 4.0 mol/L of NH$_4$Cl solution were separately injected from each inlet with a constant flow rate of 10 µL/sec. The measurement points were shown in Figure 10(c). The measurement points were set at 2-mm downstream of the junction and they located with 100 µm intervals in the spanwise direction, (i.e., $X = 50$ - 950 µm). By applying the relationships obtained in the calibration experiment to the measured intensities, the concentration at the measurement points were calculated.

Figures 11(a) and 11(b) show the concentration profiles of Na$_2$SO$_4$ and NH$_4$Cl obtained by the CARS system, respectively. The values of Na$_2$SO$_4$ concentration measured at $X = 300$-500 µm and NH$_4$Cl concentration measured at $X = 500$-700 µm are higher than those predicted by the diffusion theory. This is considered to be caused by the influence of the non–resonant background noise.
3.4 P-CARS system

When the polarization angle between CARS light and the non–resonant background noise was adjusted to 90 degrees, only the noise can be eliminated by the polarization plate. To arrange the angles of the CARS light polarization direction and polarization direction of the noise to 90 degrees, the angle between the polarization directions of two excitation beams (i.e., pump and Stokes beams) was adjusted to 60 degrees, as shown in Figure 12. For the development of the P–CARS system, λ/2 plates and a polarization plate were installed to the CARS system.

To confirm the utility of the P–CARS for the concentration measurement, a measurement in a simple flow field was conducted. Figure 13(a) shows the location of the measurement points. An I–shaped channel, whose width and depth are 1 mm, was used for the experiment. The inlet of this channel was connected to the syringe pump, and 1.0 mol/L of Na₂SO₄ solution was injected with a constant flow rate of 10 µL/sec. The concentrations at the middle of the channel were measured at 50 µm, 150 µm and 250 µm from the wall. Figure 13(b) shows the result of this measurement. The value of the concentration at 50 µm was not obtained. This is because of the optical problem mentioned in the following part.
Figure 13. (a) Schematic of the I-shaped channel and measurement points and (b) concentration measured at 50 µm, 150 µm and 250 µm from the wall.

Figure 14(a) shows the schematic of the optical path between objective lenses. In this case, since a part of excitation beams was eliminated by the edge of the channel, the measurement near the wall is impossible. Then, excitation beam diameters were changed by a pair of plano-convex lenses, in order to focus all excitation beams to the measurement point without the interference of the channel wall, as shown in figure 14(b).

Figure 14. Concepts of optical pass (a) without and (b) with plano-convex lenses.

For the P-CARS experiment, λ/2 plates, a polarization plate and a pair of plano-convex lenses were additionally installed to the CARS system, as shown in Figure 15. Polarization directions of the excitation beams were adjusted by the λ/2 plates, and the non-resonant background noise was eliminated by the polarization plate. In the following experiment, the Na₂SO₄ concentration were measured.

Figure 15. Schematic of P-CARS system.

3.5 Experiments using CARS system and Results
3.5.1 Calibration experiment using mixed solution
To confirm the noise cancellation effect by P-CARS, calibration experiments using mixed solutions were conducted. The components of the mixed solutions are shown in Table 2. These solutions consist of Na$_2$SO$_4$ as target component and NH$_4$Cl as the noise component. In this experiment, relationships between CARS light intensity ratio and the concentration of Na$_2$SO$_4$ in the mixed solutions were obtained using CARS system and P–CARS system. And the results obtained by the two systems were compared.

**Table 2. Components of mixed solutions**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Sample</th>
</tr>
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<tbody>
<tr>
<td>Condition 1</td>
<td>sample only</td>
</tr>
<tr>
<td>Condition 2</td>
<td>sample + NH$_4$Cl (2.0 [mol/L])</td>
</tr>
<tr>
<td>Condition 3</td>
<td>sample + NH$_4$Cl (4.0 [mol/L])</td>
</tr>
</tbody>
</table>

Figures 16(a) and 16(b) show the results of the calibration experiment obtained by conventional CARS and P–CARS, respectively. Blue, red and green lines are the result of Condition 1, 2 and 3 in table 2, respectively. Although calibration curves show quadratic functions in all cases, intensity of the CARS light increased as according to increase in the NH$_4$Cl concentrations in case of experiment using the CARS system. On the other hand, in the case of the P-CARS system, calibration curves of three conditions show almost the same tendency regardless of NH$_4$Cl concentration. The results indicate P–CARS successfully eliminated the non–resonant background noise.

**Figure 16. Calibration curves obtained by (a) the CARS system and (b) the P-CARS system.**

### 3.5.2 Concentration measurement in mixing flow field

According to the above calibration result, Na$_2$SO$_4$ concentration were measured in a mixing flow field. The mixing field was at the junction area of the Y–shaped channel. The detail and condition of the Y–shaped channel was the same as the channel used in the CARS experiment, shown in figure 10. By applying the relationships obtained in the previous experiment to the
measured intensities, the concentration at the measurement points were calculated. Figure 17 shows the result of the Na₂SO₄ concentration profile in the flow field obtained by P-CARS. The information near the interface between two solutions was not successfully obtained by the CARS system because of the influence of the non-resonant background noise scattered from NH₄Cl, shown in figure 11(b). However, in the case of the P-CARS system, the information of boundary between two solutions was clearly obtained. It was confirmed that P-CARS has an ability to measure the concentration at arbitrary points including near the wall and interface of solutions. Therefore, P-CARS can be effective approach for the investigation of the ion motion in micro-scale mixing flow fields.

![Figure 17. Na₂SO₄ concentration profile obtained by P-CARS system.](image)

4. Conclusions

Non-intrusive measurement techniques for the ion concentration measurement in electrolyte solutions were developed using CARS and P-CARS. A CARS system that have an ability to realize the non-intrusive ion concentration measurement was developed. Relationships between Na₂SO₄ and NH₄Cl solution concentrations and CARS light intensity ratios were obtained. The relationships show quadratic functions that agreed with the theory. Moreover, the concentration profiles of Na₂SO₄ and NH₄Cl in a mixing field in a Y-shaped channel were obtained using the relationships. However, the values of each solution concentration measured near the boundary of two solutions are higher than those predicted by the diffusion theory because of the non-resonant background noise. Subsequently, a P-CARS system was developed in order to eliminate the non-resonant background noise. Relationships between Na₂SO₄ concentration in Na₂SO₄/NH₄Cl mixed solutions and CARS light intensity ratio were obtained. These results show that NH₄Cl does not influence the calibration curves obtained by the P-CARS system, in contrast to the CARS system. Finally, a concentration profile of Na₂SO₄ in a mixing field was obtained. A comparison of the concentration gradients of Na₂SO₄ in the mixing field showed that the P-CARS system successfully eliminates the non-resonant background noise.
Acknowledgements

This work was supported by a Grant-in-Aid for Scientific Research (A) (No. 15H02223) from the Japan Society for the Promotion of Science.

References

