

Feasibility Study for the Monitoring of Urea in Dialysate Solution using Raman Spectroscopy

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We have determined the urea concentration in an aqueous solution using Raman spectroscopy by incorporating a Teflon tube as an effective intensity correction standard as well as sample container. A non-overlapping Teflon band was used as the reference peak to correct Raman intensity variations that occasionally resulted from changes in laser power. To increase the sensitivity, we positioned a copper reflector inside the Teflon tube to maximize the collection of Raman scattering. The obtained accuracy using Raman spectroscopy was 0.53 mM, close to the range of accuracy of previous NIR studies (0.15–0.52 mM).

Key Words : Urea, Hemodialysis, Intensity correction, Copper reflector, Raman spectroscopy

Introduction

With the use of NIR (near-infrared) spectroscopy, the monitoring of urea concentration during hemodialysis has been recently studied.^{1–6} Hemodialysis solution is a mainly buffered aqueous solution; therefore, the NIR feature of urea is greatly superimposed with strong absorption bands of water, eventually leading to substantially degraded signal-to-noise ratio of urea band due to large attenuation of NIR radiation by water. Simultaneously, unwanted baseline variation of urea band could be introduced due to occasional shift of water band by even small external perturbation such as minor temperature change.⁷ In addition, the molar absorptivity of urea in NIR region is low and the band is broad due to the nature of overtone and combination. Under these circumstances, for reliable and robust measurement of urea, calibration procedure especially using multivariate methods⁸ is necessary to carefully execute to properly address selectivity and sensitivity of a calibration model without overfitting.

Even with the existing hurdles as listed above, Arnold group has shown successful NIR based urea measurement by investigating critical analytical issues such as minimal noise level and optimal spectral range.¹ In addition, to ensure the selectivity under the use of broad and overlapped NIR spectral feature, the calibration based on NAS (Net Analyte Signal) was also investigated along with conventional PLS (partial least squares) regression.³ Recently, this group had developed AOTF (Acousto-Optic Tunable Filter) based NIR instrumentation for fast on-line analysis of urea concentration in dialysate.⁴ In parallel, Jensen group investigated IR (infrared) spectroscopy for the same purpose⁵ and also demonstrated dual beam FT-NIR configuration for urea measurement.⁶ In overall, the accuracies acquired in the previous NIR studies^{1–6} ranged from 0.15 to 0.52 mM.

For quantitative analysis of analytes in an aqueous matrix such as dialysate, Raman spectroscopy would be an ex-

cellent alternative method because it enables to obtain water-interference free or minimized spectral information. In addition, bandwidth of Raman peak is considerably narrower compared to that of NIR band, thereby providing the improved spectral selectivity. Simultaneously, Raman measurement is also simple and fast with capability of on-line analysis. Nevertheless, Raman spectroscopy has not readily exploited for the measurement of urea in dialysate. The concept for urea monitoring using Raman spectroscopy has been patented; however, in this patent, only five samples in the 0–450 mg/dL (0–75 mM) range were simply measured without extensive analytical investigation as well as clear justification of measurement error.⁹ Alternatively, Raman spectroscopy was used to determine the concentration of urea in urine in which its concentration (273 mM) was much higher than that normally present in dialysate solution (2–5 mM range).¹⁰

In this paper, we have attempted to determine the urea concentration in an aqueous solution using Raman spectroscopy by incorporating Teflon tube as an effective intensity correction standard as well as sample container. A non-overlapping Teflon band was used as the reference peak to follow Raman intensity variation, occasionally resulting from laser power change. To increase sensitivity of the measurement, we positioned a copper reflector inside Teflon tube to increase the collection efficiency of Raman scattering. Both an univariate calibration method using the peak area of urea as well as principal component regression (PCR) were used to determine urea concentrations.

Experimental

Sample Preparation and Spectral Collection. Fifteen different concentrations (range: 2–30 mM) of urea solutions were prepared by mixing an appropriate amount of dried urea in purified water. A WAI (Wide Area Illumination) Raman scheme (PhAT System, Kaiser Optical Systems, Ann

Arbor, MI, USA), illuminating a laser ($\lambda_{\text{ex}} = 785 \text{ nm}$) into a spot of 6 mm in diameter (total illumination area: 28.3 mm^2) was used to collect Raman spectra of samples. The detail explanation of the WAI scheme can be found in our previously published article.¹¹ A half-inch Teflon tube (perfluoroalkoxy fluorocarbon, outer diameter: 12.7 mm, inner diameter: 9.68 mm) containing urea samples was located at the focal point (focal length: 250 mm) for the spectral collection. Raman spectra were collected with an exposure time of 10 seconds for 30 scans (total 300 seconds for acquisition, resolution: 4 cm^{-1}). Triplicate spectra were collected for each sample. All of the calculations, including baseline correction, intensity correction, area calculation and principal component regression (PCR) were performed in Matlab version 7.0 (The Math-Works Inc., MA, USA).

Results and Discussion

Raman Spectral Features of Urea and Teflon. As generally known, Raman intensity would occasionally vary owing to change of laser power; therefore, it is necessary to have an internal or external standard that can follow the variation of Raman intensity to achieve a reliable quantitative analysis. As previously demonstrated,¹² we have used Teflon tube as an effective intensity correction standard and sample container. If there is at least one Teflon band that does not overlap with urea band, it can be effectively utilized to compensate for Raman intensity variation resulted from the change of laser power.

Raman spectrum of urea powder is shown in Figure 1(a). Urea has the strongest band at 1003 cm^{-1} (N-C-N stretching)

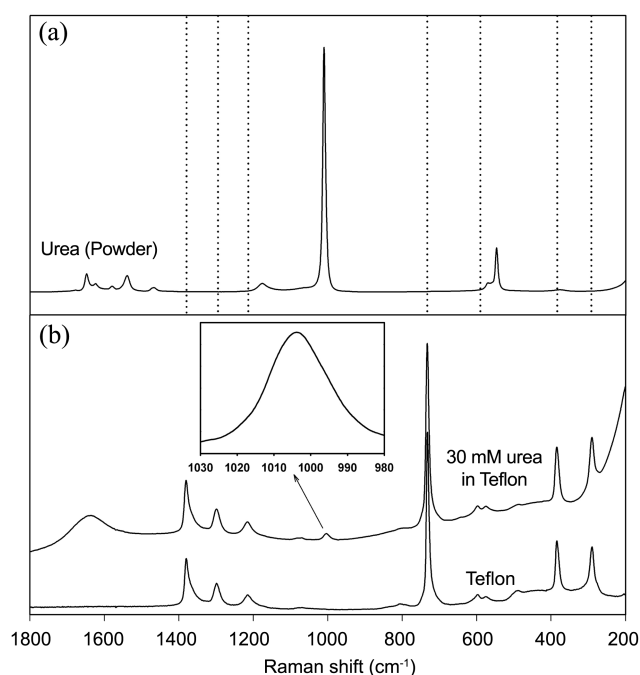


Figure 1. Raman spectrum of urea powder (a) in the $1800\text{--}200 \text{ cm}^{-1}$ range. Raman spectrum of 30 mM urea solution in Teflon tube and Raman spectrum of empty Teflon are shown (b). The urea band at 1003 cm^{-1} is magnified inside the figure.

and weak bands are also observed at 1648, 1540 and 547 cm^{-1} .¹³ The most intense band at 1003 cm^{-1} should be most valuable to use for the determination of urea concentration. The bandwidth of Raman urea band especially at 1003 cm^{-1} is much narrower in comparison with that of NIR band as shown in previous publications.¹ The dotted lines on the urea spectrum correspond to the peak positions of seven Teflon bands as shown in Figure 1(b). There are seven Teflon bands in the $1800\text{--}200 \text{ cm}^{-1}$ range; while, the 732 cm^{-1} band (the stretching mode with A_1 symmetry of CF_4) shows the highest intensity.

Raman spectrum of 30 mM urea solution in Teflon tube is also shown in Figure 1(b) and the urea band at 1003 cm^{-1} is magnified in the same figure. As expected, the spectrum is resultant from the sum of spectral feature of urea solution and Teflon tube. The urea band at 1003 cm^{-1} is clearly observed, although the corresponding intensity is substantially lower due to the low concentration compared to those of Teflon bands. The other urea bands, which have considerably lower intensity compared to the 1003 cm^{-1} band, are not clearly seen in the Raman spectrum of 30 mM urea. The most intense urea band at 1003 cm^{-1} , free of overlapping with the bands from Teflon, was solely used for the quantitative analysis and the strongest Teflon peak at 732 cm^{-1} was used as the reference peak to correct the variation of intensity.

Determination of Urea Concentration. Normally, Raman spectroscopy is not a sensitive analytical method for quantitative analysis of an analyte in a low concentration range such as sub milli-molar range; therefore, it would be beneficial to additionally incorporate a strategy that could increase Raman intensity of an analyte. In this study, rather than just spectral collection through Teflon tube, we tried to improve the sensitivity of urea measurement by collecting larger amount of isotropic Raman scattering with the use of a circular copper reflector as shown in Figure 2(a). Figure 2(b) shows the picture of the reflector positioned inside Teflon tube for Raman spectral collection. Figure 2(c) presents the schematic diagram (top view) showing laser illumination and Raman signal collection inside the reflector-incorporated Teflon tube. Laser was illuminated through a hole in the reflector and the resulting Raman scattering was collect-

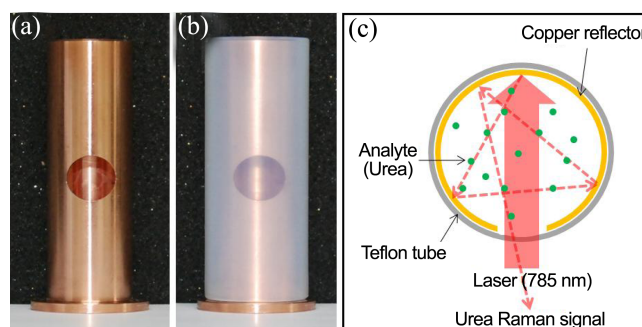


Figure 2. The pictures of circular copper reflector (a) and the reflector incorporated Teflon tube (b). Schematic diagram (top view) showing laser illumination and Raman signal collection inside the reflector-incorporated Teflon tube is also shown (c).

ed by the WAI scheme (180° back-scattering mode). Raman intensity of 1003 cm^{-1} band was increased by 3.2 fold by incorporating the copper reflector.

Initially, the variation of 1003 cm^{-1} band at 5 different concentrations (2, 6, 10, 20, 30 mM) was examined. For the comparison, the baselines of the spectra were corrected at two points (1030 and 970 cm^{-1}) and zeroed. Then, for intensity correction, the area of the Teflon band in the $780\text{--}685\text{ cm}^{-1}$ range (including the 732 cm^{-1} band) was calculated after baseline correction at 780 and 685 cm^{-1} . Finally, each baseline-corrected urea spectrum was divided by the corresponding area of the Teflon in the $780\text{--}685\text{ cm}^{-1}$ range. By this way, possible intensity variations from occasional change of laser power could be effectively corrected using the Teflon band as shown in a previous study.¹² Figure 3 shows the intensity corrected urea bands at 1003 cm^{-1} corresponding to 5 different concentrations. The 10, 20 and 30 mM urea bands are clearly observed and the intensities of urea bands increase with the concentration. The urea band of 6 mM is also clearly present; however, the spectral noise also starts to influence on the urea band. The urea band corresponding to 2 mM is barely identified, although its intensity is substantially low with the superimposition of spectral noise.

Since the urea band at 1003 cm^{-1} is distinct without overlap with those of other components, an univariate method based on the integrated area of urea band in the $1030\text{--}970\text{ cm}^{-1}$ range was utilized to determine the concentration. For the calculation of area, the baseline and intensity corrected spectra as shown in Figure 3 was used. Total of 15 samples were divided into 10 samples for the calibration set and the remaining 5 samples for the validation set. With the use of integrated area, the resulting SEC (Standard Error of Calibration) and SEP (Standard Error of Prediction) were 0.83 and 0.87 mM over the 2–30 mM concentration range, respectively. The SEP is approximately 2–6 fold higher compared to those obtained using NIR spectroscopy in the previous studies.^{1–6}

Although the urea band at 1003 cm^{-1} is selective without overlapping with other bands, the spectral collection through

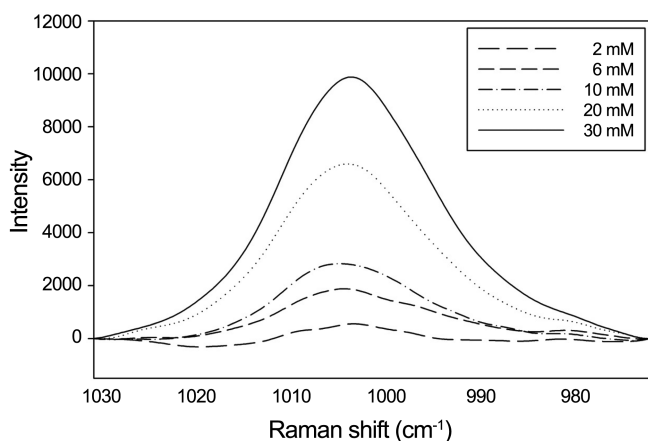


Figure 3. Intensity corrected urea bands at 1003 cm^{-1} corresponding to 5 different concentrations (2, 6, 10, 20, 30 mM).

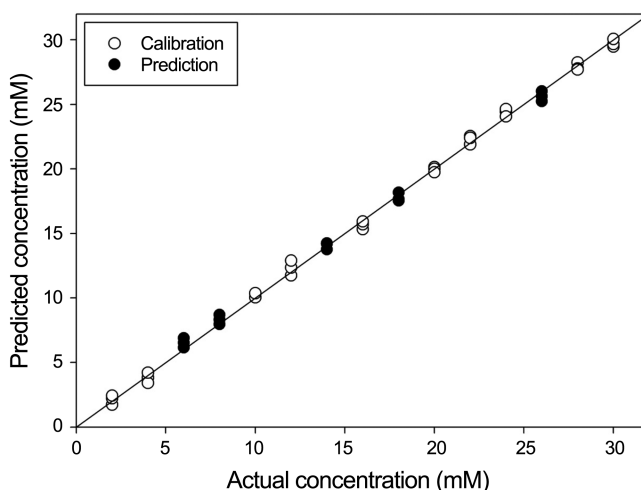


Figure 4. Concentration correlation plots for urea that resulted from the use of PCR. The open and filled circles correspond to the calibration and prediction data, respectively.

round Teflon barrier could adversely influence on the resulting spectrum, because the optical quality of Teflon is not necessarily as consistent as quartz. In this situation, the use of multivariate calibration methods would be advantageous to handle the uncontrollable spectral variations. In this study, principal component regression (PCR) regression⁸ was employed to determine the concentration of urea. The same $1030\text{--}970\text{ cm}^{-1}$ range was used for PCR. The resulting SEC and SEP were 0.54 and 0.53 mM, respectively. Two factors were used for the calibration. The corresponding concentration correlation is shown in Figure 4. The open and filled circles correspond to the calibration and prediction data, respectively. The concentration correlation is linear over the 2–30 mM range with good accuracy. The use of PCR provided the better accuracy compared to that of the univariate method (integrated area). Since the spectral variation present in the dataset was mainly from the change of urea concentration, the use of one factor would be sufficient; however, the use of one additional factor led to the better accuracy. It is presumed that the second factor is employed to model uncontrollable spectral variations in the course of spectral collection through Teflon tube. Similarly in our previous study of Raman measurement of an etchant solution directly through Teflon tubing,¹⁴ two factors were also used for the determination of hydrogen peroxide which also had a non-overlapping Raman band.

When Raman spectral collection was performed with the use of copper reflector and PCR was performed using only baseline-corrected spectra without the intensity correction using the Teflon peak, the resulting SEC and SEP were 0.89 and 0.91 mM, respectively. In addition, the intensity-corrected Raman spectra collected without use of the copper reflector (Teflon tube only) were used for PCR, the resulting SEC and SEP were 0.65 and 0.66 mM, respectively. As shown above, the use of both intensity correction and copper reflector helped to improve the accuracy for measurement of urea concentration.

Conclusion

The obtained accuracy using Raman spectroscopy is close to the accuracy range of previous NIR studies, however, it should be further improved for real practical application. While, the proposed Raman scheme has also advantages such simplicity of measurement (simple spectral collection by just directly illuminating laser through Teflon tube) and utilization of water-influence minimized urea band for quantitative analysis. The use of laser excitation with a shorter wavelength (higher frequency) will improve the sensitivity since the intensity of Raman scattering is proportional to fourth power of frequency of excitation. Dialysate sample is colorless and simple in composition, so fluorescence would not occur even with the use of shorter wavelength excitation. Simultaneously, the use of high power laser will further increase the sensitivity of urea band.

For the on-line analysis of urea concentration, the required sample volume would be too large when the proposed scheme is used. To reduce the sample volume for on-line analysis, narrow size Teflon tube, such as 1.5 mm O.D. (Outer Diameter), can be easily used. Since the illumination area of the WAI scheme is large, a single tube can be arranged to be in parallel under the illumination spot. By this approach, the larger volume of sample can be illuminated, although a narrow tube is used. As performed in this study, a proper reflecting scheme positioned behind of the tubes would also help to increase the resulting Raman intensity.

The above-mentioned strategies to improve the sensitivity of Raman measurement for monitoring urea in dialysate are under investigation. Additionally SERS (Surface enhanced Raman scattering) based on-line measurement will be also

considered.

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