Intensity Correlation Analysis of Raman Spectra of Concentrated Ficoll Solutions

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ABSTRACT

The intracellular environment is crowded with diverse biomacrolecules (~80-400 mg/ml), likely affecting various biological processes such as protein folding, binding of small molecules, enzymatic activity, and pathological protein aggregation. As a model we have been using solutions of Ficoll, a highly branched polysaccharide, to mimic the environment. Besides its biomedical applications (e.g. blood separation), it has been used as a macromolecular crowder in studies of protein folding and stability, cell volume signaling, tissue engineering, and nanotransport. In this study, our goal is to identify and assess Raman spectral signatures associated with Ficoll molecules and Ficoll-Ficoll interactions for future investigations of crowding effects.

In addition to the Raman peaks of water (~1640 cm^{-1} and ~3200 cm^{-1}) and dissolved O_2 (~1556 cm^{-1}) and N_2 (~2331 cm^{-1}) we identified a distinct Raman peak (~2900 cm^{-1}) in the 1500-3500 cm^{-1} wavenumber range, which is associated with Ficoll and CH and CH_2 stretching modes. As the Ficoll concentration increases, the intensity of the Ficoll Raman peaks increases while the intensity of the water Raman peaks decreases, the latter likely due to reduction of water content. Further, we have applied the intensity correlation analysis (ICA) method to assess systematic changes of Raman spectra with Ficoll concentration (up to 1000 mg/ml). ICA indicates an overall linear trend over the full wavenumber range, but also shows closed loops that can be attributed to slight changes of the profiles of certain peaks. The results demonstrate ICA as a potential insightful tool for identifying Ficoll in chemical analysis of crowded biological samples.

Keywords: Raman Spectroscopy, Ficoll, Intensity Correlation Analysis, CH stretching, OH stretching, HOH bending.

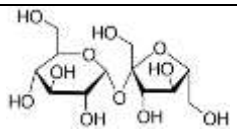
1. INTRODUCTION

Raman spectroscopy has been applied as a powerful analytical technique for optical detection and characterization of molecular structures of diverse samples [1,2]. Typically, it uses a laser beam (e.g. wavelength 780 nm) to illuminate a sample and excite molecules that scatter light at different wavelengths. Experimentally, the scattering at the same laser wavelength (Rayleigh scattering) is blocked and the intensity distribution of the rest of the scattered radiation is measured as a function of wavelength, providing a versatile and simple tool for in-situ rapid spectrochemical analysis of various types of chemical compounds in the samples in a laboratory as well as outdoor settings. Raman spectrum is associated with the vibrational modes of the scattering molecules, and analysis of the measured spectrum is conducted to determine the observed peaks, which are then attributed to the complex structure of the studied molecules and the possible effects of the host medium and inter-molecular interactions – the challenging task. In this context, it has been recognized that macromolecular crowding -the addition and presence of macromolecules- can play significant role on various biochemical and biophysical processes, including polymerization/depolymerization, gene expression, protein trafficking, intracellular signaling, anomalous diffusion, and structural changes [3-13].

In this paper, we report the results of analysis of Raman spectra measured on Ficoll solutions prepared at different concentration. Our goal is to assess possible effects of the concentration increase on the Raman spectra. Table 1 provides information about the Ficoll polysaccharide (~70kDa). Ficoll 70 is a highly-water soluble, neutral,

biocompatible, branched sucrose-polymer with an average molecular weight of 70 kDa. It is formed by copolymerization of sucrose with epichlorohydrin [14]. For *in vitro* studies, it has been widely used as a

Table 1: Characteristics of Ficoll molecule.

Name	Molecular Formula	Molecular Structure	Molar mass
Ficoll	(C12H22O11)n.(C3H5ClO)n		70,000

macromolecular agent to investigate the effects of crowding on the behavior of various molecules and biomacromolecules in solutions. In applications, it has been used, for example, as a standard in laboratories for the isolation of human lymphocytes because it is uncharged and relatively inert [15-16].

To assess changes or differences of the measured Raman spectra we applied the intensity correlation analysis (ICA) method [17-20], from which we determine the Pearson's correlation coefficient. ICA is commonly applied to assess overlap of multi-channel microscopy images, in which each channel measures the intensity of a specific item (e.g. fluorescent proteins) and the colocalization is determined at pixel level. For example, if two proteins are labeled with two different fluorescent probes and dispersed in the sample, we first measure images of the sample in two different color channels, each being associated with the fluorescence characteristics of one of the probes. Then, the goal is to assess the extent of geometrical pixel colocalization of the fluorescent signals and intensities from the two different images, reflecting the extent of colocalization of the proteins. That is, by determining the overall overlap of the two different intensities measured at the same pixel level (sometime close pixels) from the two-color channels. We extend ICA to the one-dimensional Raman spectra and determine the extent of overlap of two spectra along the wavenumber. The intensity-intensity plot from two separate spectra is then analyzed to determine and quantify the colocalization and symmetry of the various peaks via the Pearson's coefficient.

2. EXPERIMENTAL METHOD

2.1 Sample preparation

Ficoll solutions were prepared by dissolving Ficoll powder into Deionized (DI) water (up to 1000 mg/ml). The solutions were sonicated at room temperature to ensure complete dissolution and homogeneity, and for high concentration (> 500 mg/ml), the solutions were further left overnight before measurements. Samples of Ficoll solutions were loaded in pre-cleaned quartz cell holders that have low Raman spectrum background. As a control experiment, water samples were also used.

2.2 Raman Spectrometer and Spectra Collection, Data Analysis

We used a HORIBA MacroRAM spectrometer equipped with a 785-nm diode pumped solid-state laser for exciting the molecules and a Syncerity back-illuminated NIR CCD for detection. The spectrometer, which covers a spectral range between 100 and 3400 cm^{-1} and has 42,550:1 dynamic range, comes with a LabSpec6 software package for measurements and analysis. For further processing of the data, plotting, and analysis we used Kaleidagraph software package.

2.3 RESULTS

Figure 1 shows measured Raman spectra from samples of water and Ficoll solutions prepared at different concentrations (100, 300, 500, 1000 mg/ml). The spectra were taken at room temperature and we display 1500-3500 cm^{-1} wavenumber range. The spectra show different peaks associated with water (~1600 cm^{-1} due to HOH bending; ~3200 cm^{-1}due to OH stretching modes), dissolved nitrogen molecule (N_2 ~2320-2340 cm^{-1}), dissolved oxygen (O_2 ~ 1560 cm^{-1}), and a peak associated with Ficoll molecule and likely due to CH and/or CH_2 stretching (~2900 cm^{-1}) [1,2,21,22]. Note the systematic increase of the intensity of the 2900-peak with the increase of the Ficoll concentration. Meanwhile, the intensity of the two peaks, 1600-peak and 3200-peak, shows systematic decrease likely due to systematic reduction of the amount of water. That is, the more Ficoll molecules, the less water molecules.

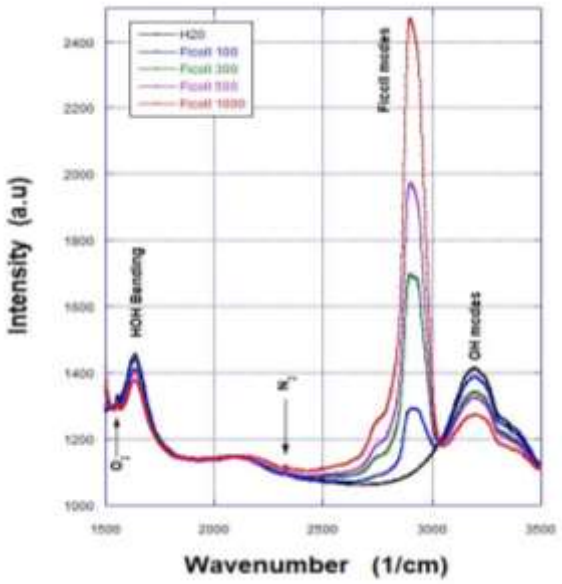


Figure 1: Raman spectra of water and Ficoll solutions prepared at different concentrations. Inset indicate the Ficoll concentration. The peaks are labeled with the associated vibrational modes.

For further insight, we show in Figure 2 changes of the intensities of both of 3200-peak of OH modes and 1600-peak of HOH bending as a

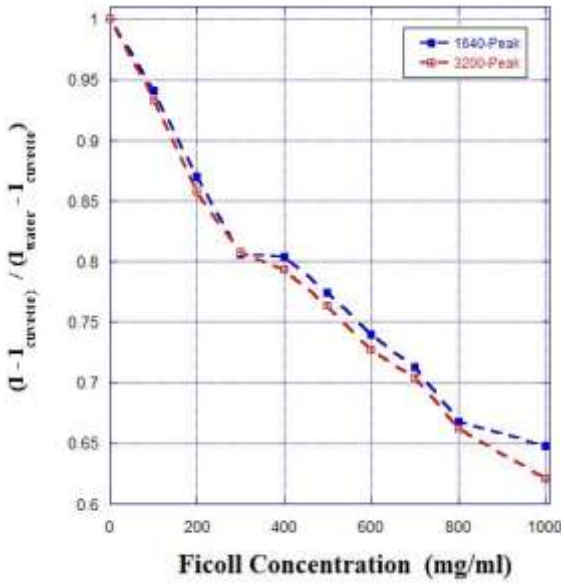


Figure 2: Decrease of intensities peaks associated with water molecules: ~1640 cm^{-1} of HOH bending and ~3200 cm^{-1} associated with OH stretching when Ficoll concentration is increased. The intensities are corrected for the cuvette contributions. The inset indicates the corresponding peaks associated with water.

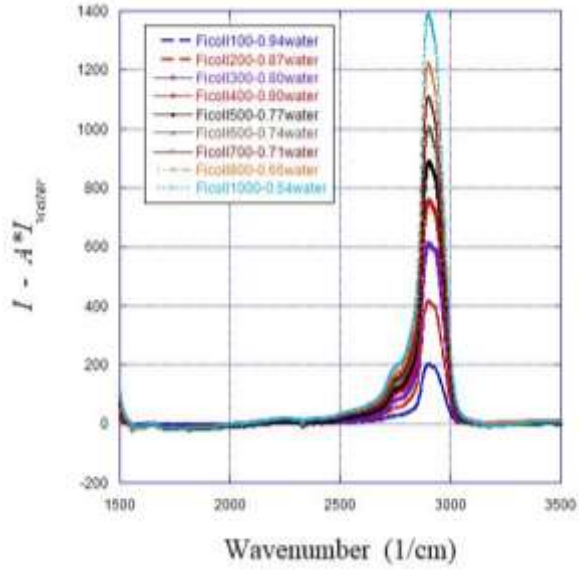


Figure 3: Ficoll peak at ~2900 cm^{-1} associated with CH stretching modes are plotted as a function of wavenumber. Each intensity distribution was corrected for the appropriate contribution of water and cuvette as background (see text). Inset indicates the Ficoll concentration and the value of the proportional coefficient, A

function of Ficoll concentration. Here, we note that we subtracted the background contribution from the cuvette, which is common to all samples. And as such the plot shows both peaks decrease and track each other, providing a way to subtract the appropriate background due to water in each concentration-dependent spectrum. That is, we did not use the spectrum from the water and cuvette as background. Rather, we first subtracted the cuvette-background contribution on all spectra of Ficoll solutions and water. Then, we subtracted a proportional contribution of the cuvette-corrected water spectrum according to the plot shown in Figure 2. In Figure 3 we show the 2900 peak of the Ficoll solutions prepared at different concentrations. Under this procedure most of the water contribution to Ficoll spectra appear to be flattened.

To assess possible effect of Ficoll concentration on the Raman spectra of Ficoll molecule -more specifically on the CH and/or CH_2 stretching peak- we proceeded to apply the intensity correlation analysis method, in which we plot the intensity of the spectral peak measured at different concentrations (Figure 3) against that of the lowest concentration [Ficoll]=100mg/ml. In Figure 4 we show the intensity-intensity plot, which indicates overwhelmingly linear trends in the intensity-intensity plot. This linearity is indicative of little or no measurable effect of the Ficoll concentration on the intensity profiles within the experimental limits. An exception could be the case of [Ficoll]=1000 mg/ml concentration, where there appears a closed loop in the intensity-intensity plot, likely due to a slight shift of the peak but without major change of the width.

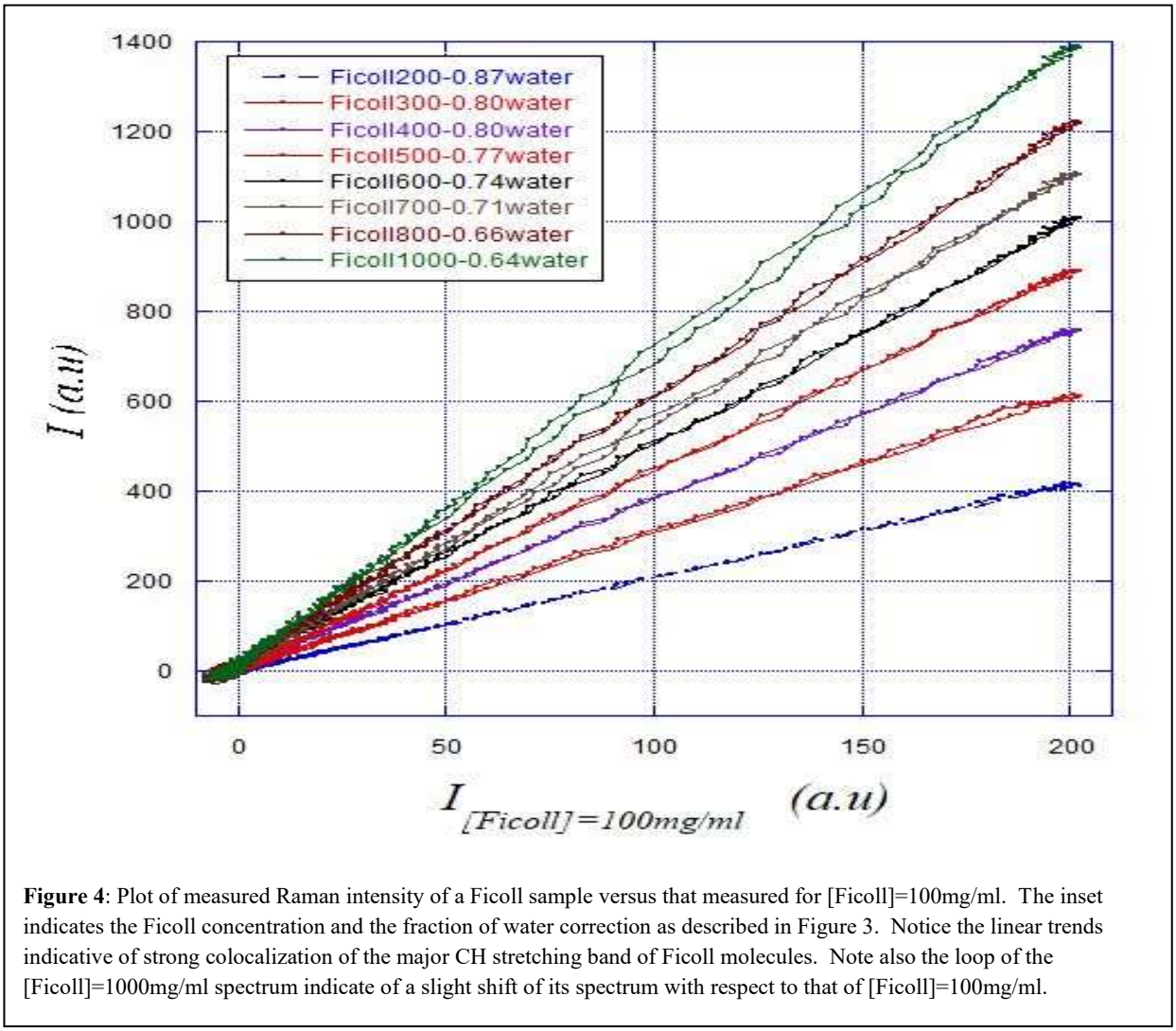


Figure 4: Plot of measured Raman intensity of a Ficoll sample versus that measured for [Ficoll]=100mg/ml. The inset indicates the Ficoll concentration and the fraction of water correction as described in Figure 3. Notice the linear trends indicative of strong colocalization of the major CH stretching band of Ficoll molecules. Note also the loop of the [Ficoll]=1000mg/ml spectrum indicate of a slight shift of its spectrum with respect to that of [Ficoll]=100mg/ml.

3. CONCLUSIONS

Application of Raman spectroscopy for the detection, identification, and characterization of biomacromolecules in a macromolecularly-crowded environment is a challenging proposition as one needs to identify specific and integrative spectral signatures from their basic structures and vibrational modes. Further, one would seek to elucidate the intricate effects of crowding due to systematic addition of biomacrolecules such as polysaccharides, proteins, and DNAs. As a biomacromolecular crowder we have been using Ficoll, a branched polysaccharide molecule, with well-known chemical and physical properties, especially high solubility in water. Here, we measured Raman spectra of Ficoll samples of solutions prepared at different concentrations and focused on determining possible Raman signatures of Ficoll molecules (see Table 1 for the structure). In the 1500-3400 cm^{-1} range we observed several peaks and assigned them to specific vibrational modes. In contrast to the typical background correction in dilute solutions, we applied a systematic approach to correct for the water-cuvette background by using two distinctive peaks of water (~1600 cm^{-1} and ~3200 cm^{-1}; see Figure 2) to monitor the water content at each Ficoll concentration and to provide a means for estimating the appropriate background signal due to water content in the high-concentration regime solutions (up to 1000 mg/ml). For the 2900-peak, which is attributed to Ficoll CH and CH_2 stretching, Figure 3 shows well-corrected background. Subtraction of all 100% water background would have dipped the OH and HOH peaks in the unphysical negative intensities. Further, Figure 4 demonstrates how ICA method can be readily applied to determine correlation or not among the measured spectra. That is, whether increase of Ficoll concentration may affect the overall peak profile, including width and shift. Figure 4 indicates overall linear correlations among the 2900-peak profiles of the Ficoll solutions with close to R=0.999 correlation. However, the appearance of a loop in the case of [Ficoll]=1000mg/ml reveals possible small shift of the overall peak compared to that at much low concentration, [Ficoll]=100mg/ml. Altogether, the present results demonstrate how ICA can quantify possible changes of the Raman spectrum of biomacromolecular system due to the host matrix

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