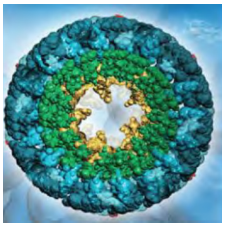
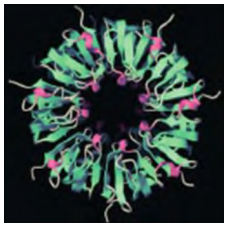
HORIBA Scientific

Raman Spectroscopy

Assessing biotherapeutics stability using Raman spectroscopy





Application Note

Life Sciences RA79

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Abstract: For close to 20 years the pharmaceutical industry has been producing biotherapeutic drugs as they offer targeted drug efficacy with minimal side-effects. Responding to the need for self-administered drugs, protein-based therapeutics have to be delivered at high concentrations. However, the tendency for proteins to aggregate in solution is increased at these high levels (>100 mg/mL). As a result, the formation of aggregates can alter protein structure which can, in turn, influence the bioavailability of the drug, can induce immunogenic reactions, and can even cause thrombolytic events. We have measured solutions of lysozyme under conditions known to effect its physical state in order to investigate the potential of Raman spectroscopy as a non-invasive and label-free tool to assess protein formulation stability. Results from this study identified specific Raman signature bands in this protein that can be used to identify individual amino acid residues that are reflect structural changes in proteins.

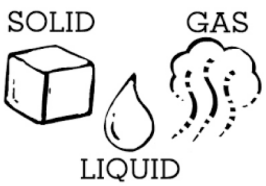
Keywords: Life Sciences, Pharmaceutical, Biotherapeutics, Protein, Lysozyme, Raman vibrational bands.

Introduction

Raman spectroscopy is a light scattering technique, and can be thought of in its simplest form as a process where a photon of light interacts with a sample to produce scattered radiation at different wavelengths. The frequency difference between the incident and the scattered light characterizes the molecular vibration.

Advantages:

- No sample preparation is required.
- Water Raman signal is weak and can be subtracted from the spectra.
- Solids, liquids and gases can be measured in glass, quartz and plastic containers.



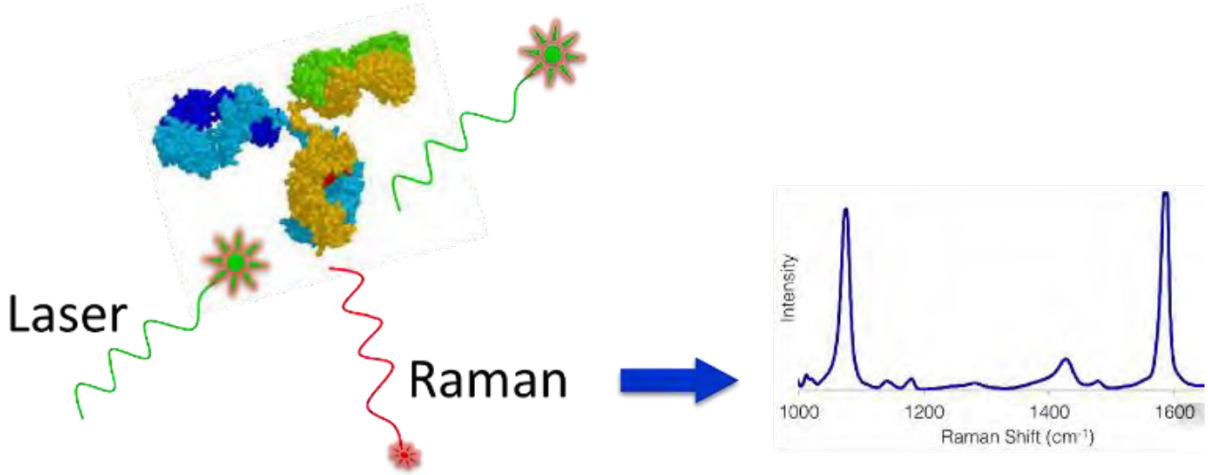


Figure 1: Raman spectroscopy principle

Objectives

Investigate the potential of Raman spectroscopy to assess the stability of protein formulations.

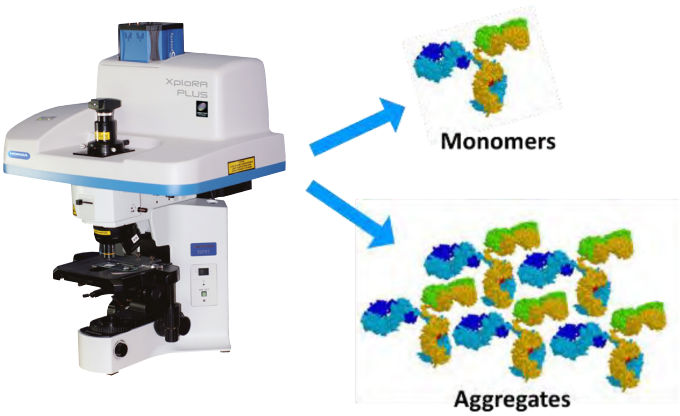


Figure 2: Monomers and aggregates measure using XploRA Raman microscopy

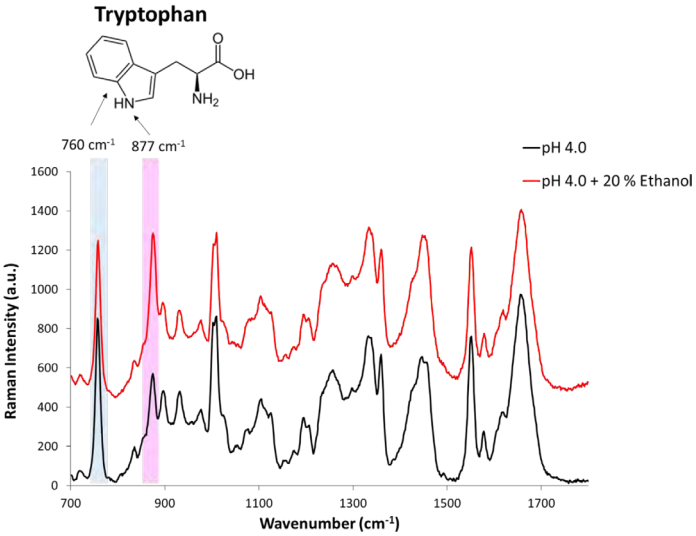


Figure 4: Raman Analysis of lysozyme (200 mg/mL) in 20 mM Citrate-PBS buffer at pH 4.0 before and after the addition of 20 % ethanol. Sample was excited with a 532 nm laser and a grating of 1800 lines/mm on the XploRA, a 200mm focal length instrument.

Results

I. Ethanol Effect

Sample	I_{877}/I_{760} Ratios	Raman Bands
pH 4.0	0.60	760 cm^{-1} band (indole ring of Trp)[3]
pH 4.0 + 20% Ethanol	1.02	877 cm^{-1} band (N_1-H site of the indole ring)[3]
Increase in relative intensities of the I_{877}/I_{760} ratios suggests an environmental change around the Trp moiety as a result of the addition of ethanol.		

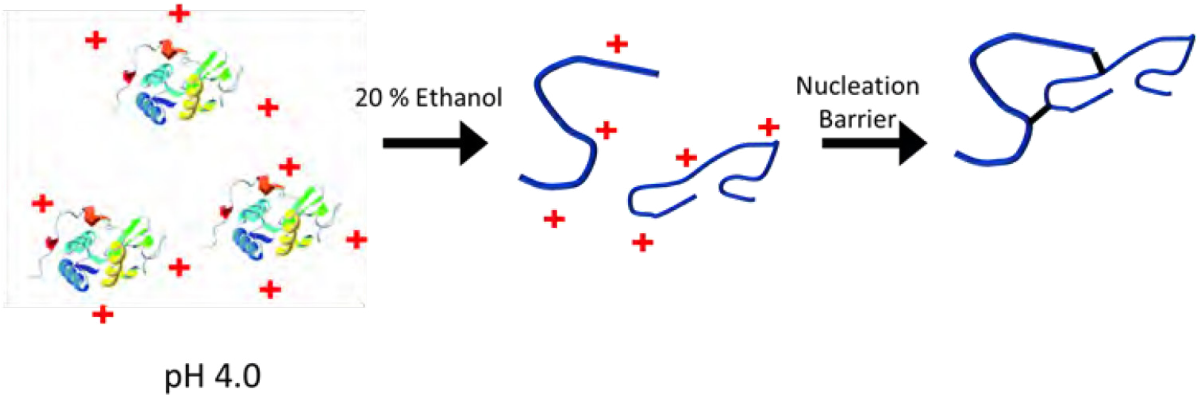


Figure 3: Ethanol effect

II. pH Effect

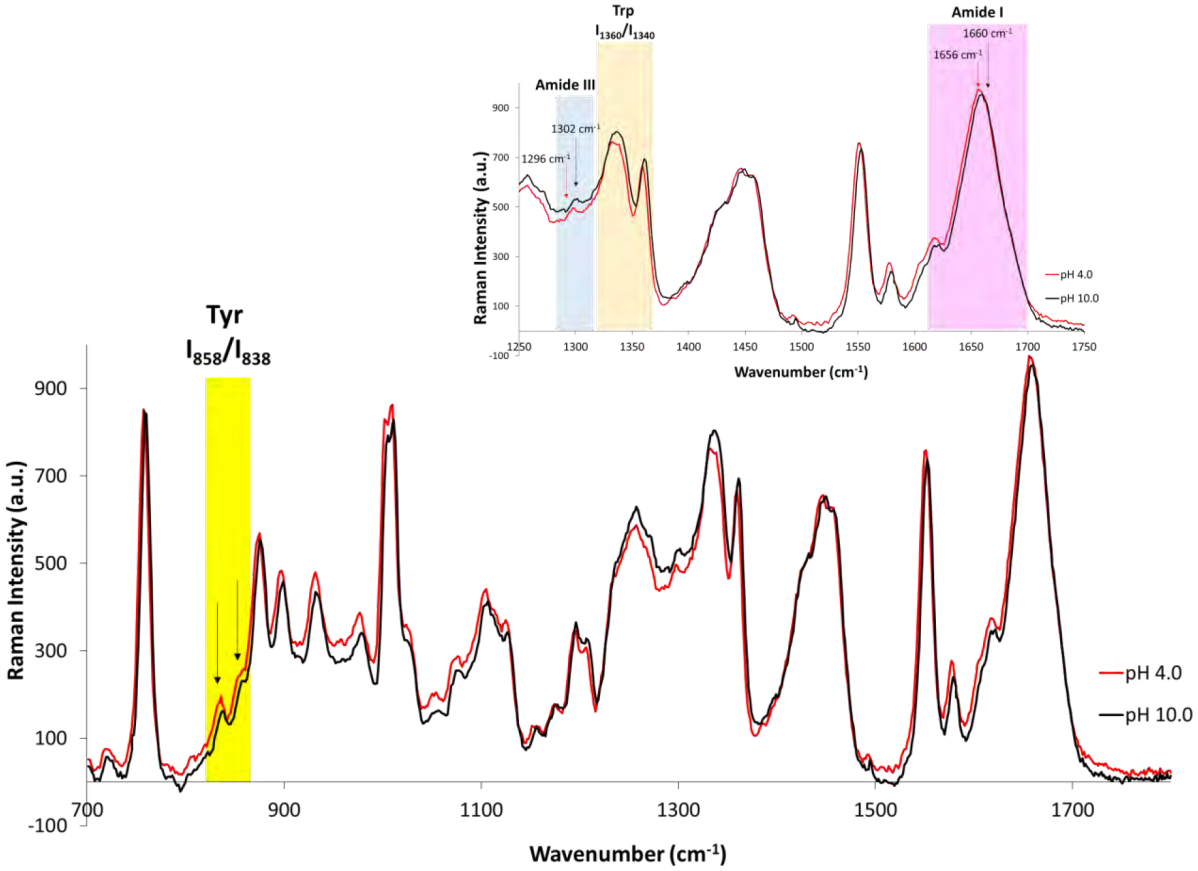


Figure 5: Raman Analysis of lysozyme (200 mg/mL) in 20 mM Citrate-PBS buffer at pH 4.0 and pH 10.0. Sample was excited with a 532 nm laser and a grating of 1800 lines/mm.

Sample	Tyr (I_{858}/I_{838}) Ratio	Amide III	Trp (I_{1360}/I_{1340}) Ratio	Amide I
pH 4.0	1.32	1296 cm^{-1}	0.89	1656 cm^{-1}
pH 10.0	1.44	1302 cm^{-1}	0.86	1660 cm^{-1}
Analysis	Increase in ratio signifies Tyr is more accessible to aqueous environment due to structure reorganization[1,2]	Shift in bands suggests a transition from α-helical to β-sheets[1,2,4]	A decrease in ratio implies that Trp is more accessible to aqueous environment[1,2]	Shift in bands suggests a transition from α-helical to β-sheets[1,2,4]

II. Concentration Effect

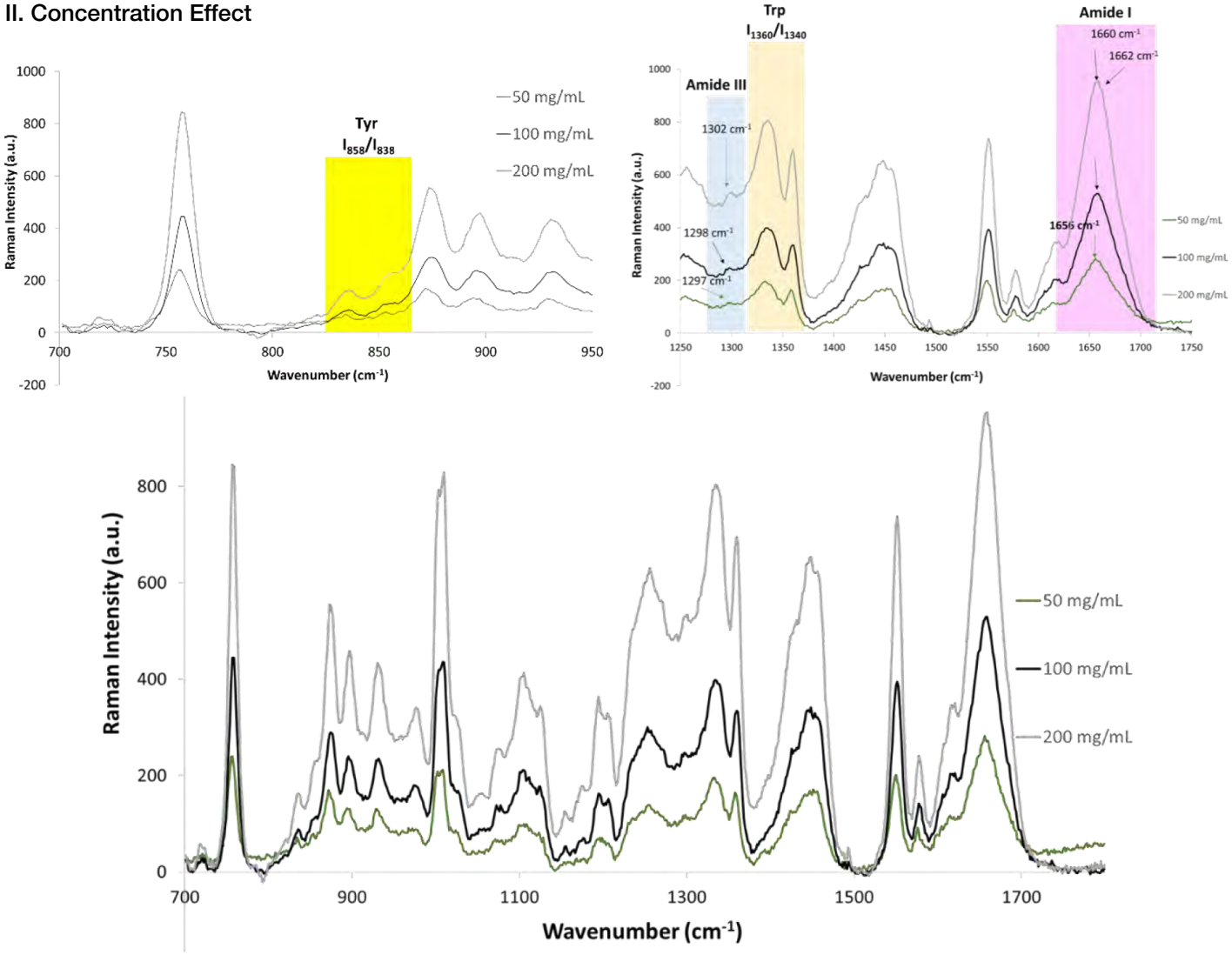


Figure 6: Raman Analysis of lysozyme (200 mg/mL, 100 mg/mL and 50 mg/mL) in 20 mM Citrate-PBS buffer at pH 10.0 . Sample was excited with a 532 nm laser and a grating of 1800 lines/mm.

Sample	Tyr (I_{858}/I_{838}) Ratio	Amide III	Trp (I_{1360}/I_{1340}) Ratio	Amide I
50 mg/mL	1.04	1296 cm^{-1}	0.81	1656 cm^{-1}
100 mg/mL	1.28	1298 cm^{-1}	0.84	1660 cm^{-1}
200 mg/mL	1.44	1302 cm^{-1}	0.86	1660 cm^{-1}, 1662 cm^{-1}
Analysis	Increase in ratio signifies Tyr is more accessible to aqueous environment due to structure reorganization[1,3,4]	Shift in bands suggests a transition from α-helical to β-sheets[1,2,4]	An inecrease in ratio implies that Trp is well buried and closer to neighboring proteins.[1,2]	Shift in bands and appearance of a shoulder peak at 1662 cm^{-1} suggests a transition from α-helical to β-sheets[1,2,4]

Conclusion

- Raman allows for the assessment of folding and unfolding processes in proteins.
- Important structural information can be attained from specific Raman vibrational bands as: amide I, amide III, Trp and Tyr bands.

Acknowledgement

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References

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