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Raman

Quantitative Analysis Using Raman Spectroscopy in Pharmaceutical Applications

Application Note

Pharmaceuticals
RA83

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Abstract: Raman spectroscopy is well known as a powerful analytical method for qualitative chemical analysis. Less well known is that under certain conditions Raman spectroscopy can also be an effective method for quantitative analysis. Here we demonstrate that capability for quantitative analysis under a variety of conditions involving different solutes in a solution and solution mixtures, and its applications to pharmaceutical analysis, such as content uniformity.

Keywords: Raman spectroscopy, quantitative analysis, calibration curve, dissolution study, MacroRAM, macroRaman

Introduction

Raman spectroscopy is generally recognized as a powerful analytical technique that is used to study the chemical composition of materials within the context of qualitative analysis. Nevertheless, under certain conditions Raman spectroscopy can also be an effective method for quantitative analysis. With liquid and solution samples, Raman spectroscopy allows one to perform rapid, non-destructive, quantitative analysis with minimal sample preparation. Raman spectroscopy provides direct chemical information about analytes in addition to quantitative information. Furthermore, the development of compact, low-cost, benchtop Raman spectrometers has made it possible to perform such analyses in virtually any locations from an undergraduate chemistry lab to an industrial environment.

Here, we have used Raman spectroscopy to perform quantitative analysis of a simple solution consisting of one solute dissolved in water, an azeotropic mixture of two liquids (water and ethanol), and a complex solution consisting of multiple solutes dissolved in a buffer. We show that Raman spectroscopy can be used to produce calibration curves to predict the concentration of complex solutions, which can then be used to correctly predict concentrations (or dosages) of APIs (active pharmaceutical ingredients) in pharmaceutical products (e.g. Excedrin®, NyQuil® and DayQuil®).

The Raman spectroscopic data in this work was collected with a MacroRAM™, a benchtop Raman spectrometer, and all data processing and analysis was done with the LabSpec 6 Spectroscopy Suite.

1. A Simple Solution

In a previous work, (1) we discussed the development of a calibration curve for quantitative analysis using Raman spectroscopy using a simple solution of one solute (guanidine hydrochloride) and one solvent (water). Raman spectra were collected from guanidine hydrochloride at the following concentrations: 0.25, 0.50, 1.0, 2.0, 4.0, 6.0, and 8.0 M (Figure 1). The Raman band at 1011 cm^{-1} was selected as a marker band to represent guanidine hydrochloride. The marker band was fitted with a G+L (Gaussian+Lorentzian) curve, and the peak area was calculated. The peak areas averaged from three sets of measurements were plotted as a function of the concentration. As shown in Figure 1 inset, a linear dependence of the peak area on the concentration was obtained from the measurements, with reproducible peak area values for each concentration. The standard deviation amongst the three sets of data was found to be extremely small compared to the difference in value between data points, thus yielding a reliable calibration curve.

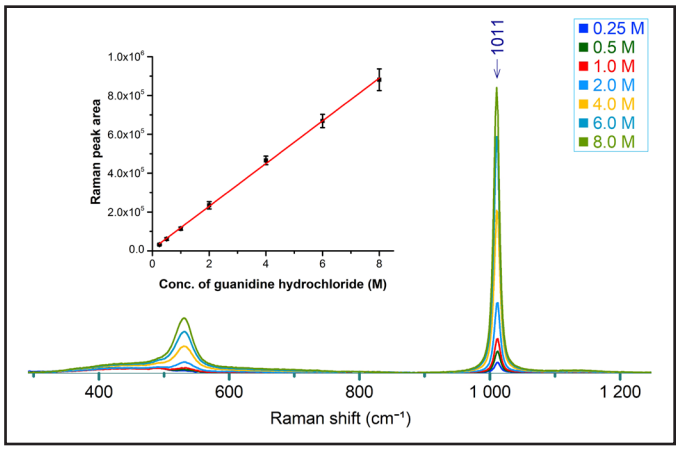


Figure 1: Raman spectra from 0.25, 0.50, 1.0, 2.0, 4.0, 6.0, and 8.0 M guanidine hydrochloride. Inset shows a calibration curve between 1011 cm^{-1} peak area and the concentration.

In this paper, we expand the discussion to more complex solutions and mixtures.

2. Another simple solution

We dissolved acetaminophen in pH 2.4 HCl at 37 ºC and prepared a series of solutions at concentrations 0.5 - 2.5 mg/ml. Raman spectra were recorded in triplicate measurements. The Raman band at 1330 cm^{-1} was selected as a marker band. The peak area of the marker band was measured after baseline subtraction and plotted as a function of the concentration. As can be seen in Figure 2, a linear dependence of the peak area on acetaminophen concentration was observed.

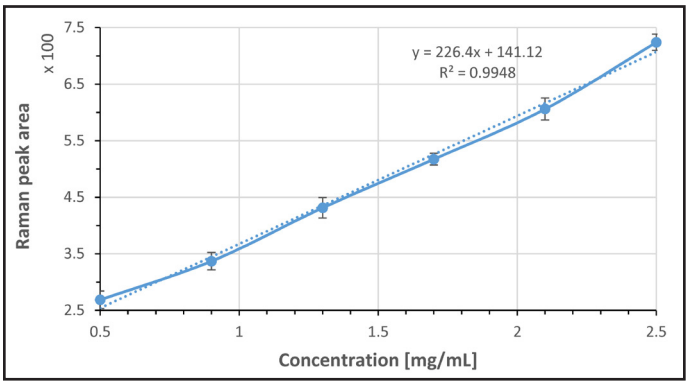


Figure 2: Peak area of the marker band at 1330 cm^{-1} of acetaminophen was used to develop a calibration curve over 0.5 – 2.5 mg/ml range. Each data point was averaged from 3 measurements and error bars indicating standard deviation are shown, as well as linear trendlines (dotted) along with line equations.

3. Application of Calibration Curve

We used DayQuil™ Cold & Flu, and NyQuil™ Cold & Flu liquid dosages as model systems to apply the calibration curve. DayQuil™ Cold & Flu contains 650 mg of acetaminophen in 30 ml dose. NyQuil™ Cold & Flu contains 325 mg of acetaminophen in 15 ml dose. (2) Three aliquots of each dosage were diluted 10 times before measuring Raman spectra (Figure 3). The concentration of diluted dosage is expected to be ~ 2.17 mg/ml.

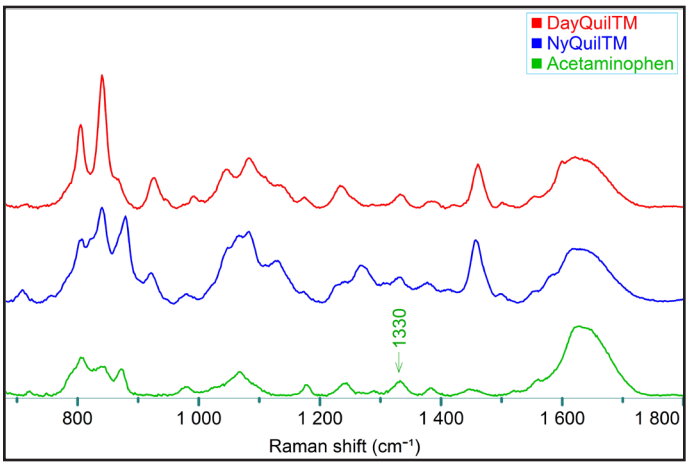
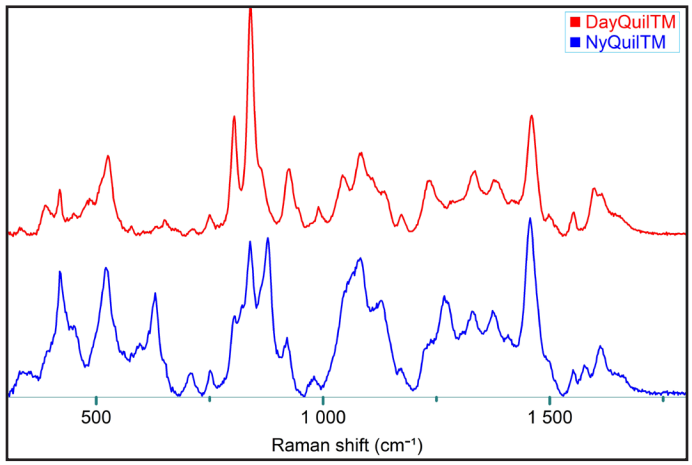


Figure 3: Raman spectra of DayQuil™, NyQuil™, and acetaminophen (Sigma-Aldrich, 2.5 mg/mL in HCl)

Raman spectra of DayQuil™ and NyQuil™ dilutes were recorded with an immersion probe (Figure 4), mimicking the measurement method of a mixing or a dissolution batch.



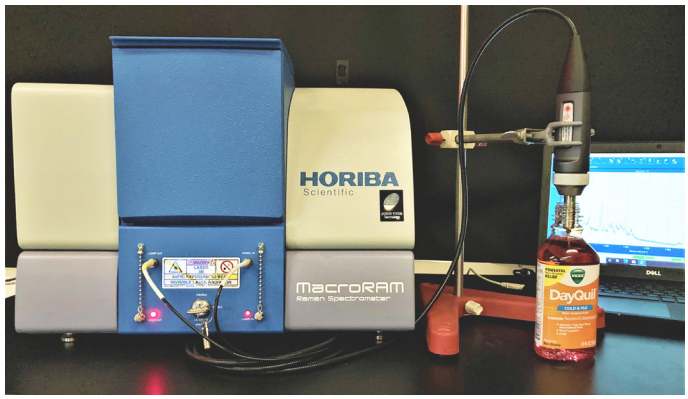


Figure 4: Raman spectra of DayQuil™ and NyQuil™ collected with an immersion probe fiber-coupled to the MacroRAM™ Raman spectrometer, and a picture of the immersion probe setup.

Using the calibration curve developed (Left, Figure 2) and the peak areas of Raman bands at ~ 1330 cm^{-1} in DayQuil™ and NyQuil™ Raman spectra, acetaminophen concentrations were predicted as 2.37 mg/ml and 1.91 mg/ml, respectively. Please note that these results are from univariate analysis using a peak area, and based on a calibration curve developed from a pure acetaminophen.

4. A Complex Mixture

Raman spectroscopy can also be used for quantitative analysis of complex mixtures such as water and ethanol mixture. Water and ethanol, when mixed, result in a volume contraction (volume of mixture < sum of volumes of components) due to greater hydrogen bonding. The degree of contraction depends non-linearly on the ratio between individual components. This complicates the calibration curve.

The mixtures were made with ethanol wt/wt (%)[1] of 0, 10, 30, 50, 70, 90, and 100%. Raman spectra were recorded at each percentage, and the data was averaged from 5 repetitions (Figure 5). The 884 cm^{-1} peak from ethanol was selected as the marker band for ethanol (Figure 5 inset), and the peak area was calculated using univariate analysis. The peak areas were then plotted as a function of the ethanol wt/wt (%), ethanol vol/vol (%)[2], and ethanol molarity[3] (Figure 6).

[1] (ethanol weight) / (weight of the mixture), [2] (ethanol volume) / (volume of the mixture), [3] (moles of ethanol) / (volume of the mixture)

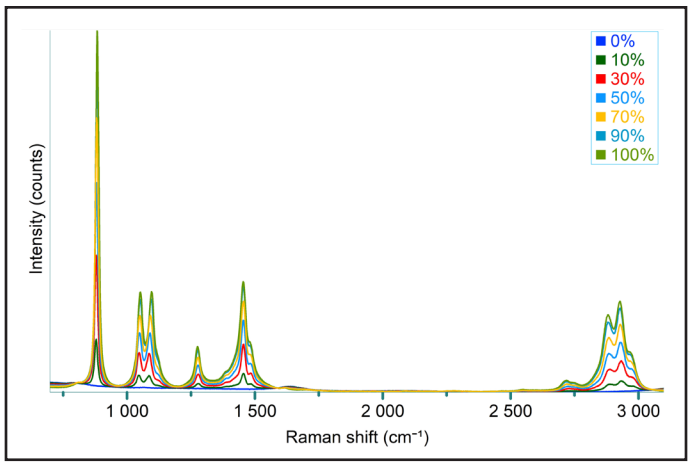


Figure 5: Raman spectra recorded from a series of ethanol-water mixtures, with ethanol wt/wt (%) percentage ranging from 0 to 100%. The peak area of the 884 cm^{-1} ethanol peak (inset) was used for building a calibration curve.

A linear relationship is often assumed between the concentration and the spectrum intensity. However, it is not always true for complex solutions with multiple solutes and mixtures like ethanol and water. The relationship, and thus the calibration curve, vary depending on the concentration unit, which is often chosen for the convenience and convention.

Peak areas of the ethanol marker band show a linear relationship with respect to ethanol concentrations in vol/vol (%) (Figure 6a) and molarity (Figure 6b), resulting in linear calibration curves. The relationship with respect to wt/wt (%) is closer to the 2nd order polynomial (Figure 6d) than linear (Figure 6c). This is because the volume contraction of the mixtures is non-linear. (3) (4) The volume contraction is greater when the wt/wt (%) are closer to 50% (e.g. 30%, 50% and 70%) than purer liquids (e.g. 0%, 10%, 90%, 100% ethanol). This demonstrates that reliable calibration curves can be produced using Raman spectroscopy even for more complex solutions and mixtures, and those calibration curves may be complex accordingly. Similar relationship between the micro-environment of a particular molecular group and its vibrations have been reported. (5)

5. A Complex Solution

Raman spectroscopy can also be used for quantitative analysis of complex solutions containing multiple solutes in solution. We prepared stock solutions by dissolving aspirin, acetaminophen and caffeine in pH 2.4 HCl at 37 ºC to achieve ~ 3.5 mg/ml by adding sodium lauryl sulfate (SLS). We prepared three equivalent sets of solutions by preparing three stock solutions (Table 1), and then diluting each stock solution in succession.

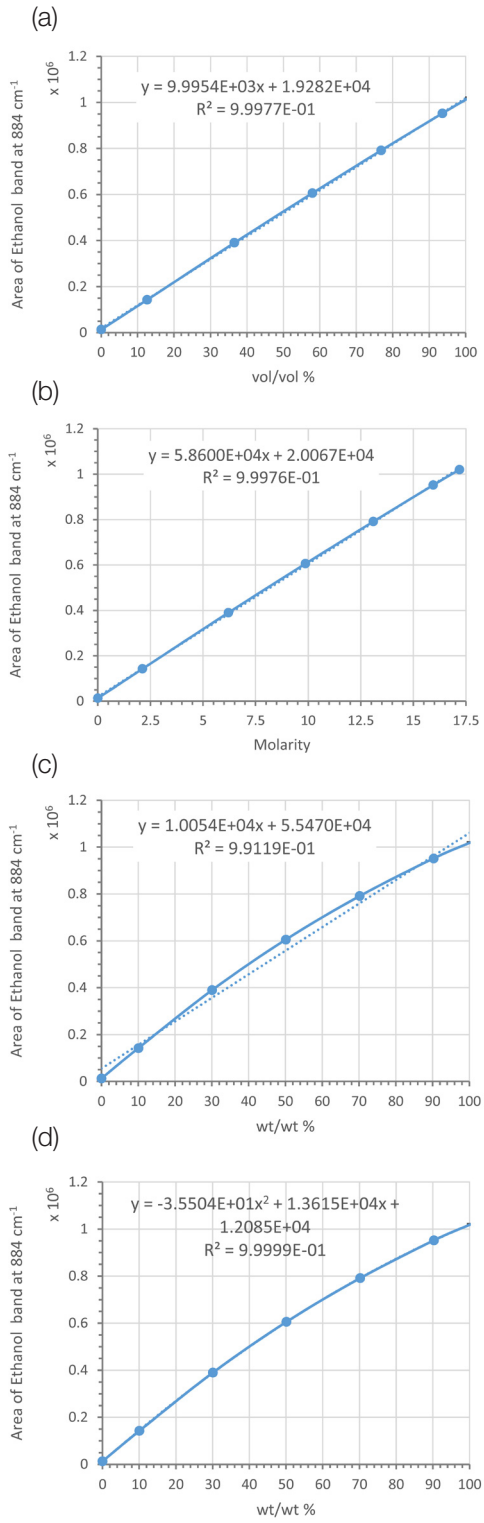


Figure 6: Calibration curves using the marker band of ethanol at 884 cm^{-1} and different concentration units: (a) vol/vol % and (b) molarity; (c) and (d) wt/wt %, Each data point was averaged from 5 measurements. Error bars indicate standard deviations. Dotted lines are trend-lines. Their equations and R^2 values are shown in the plot.

	Aspirin (g)	Acetaminophen (g)	Caffeine (g)	SLS (g)	HCl (ml)	Total (g)	Total (ml)
STOCK I	0.0264	0.0262	0.0264	0.0158	7.500	7.7146	7.60 ml
STOCK II	0.0258	0.0257	0.0258	0.0152	7.371	7.4537	7.45 ml
STOCK III	0.0267	0.0270	0.0269	0.0156	7.628	7.7095	7.70 ml

Table 1: Stock solution formulation

Raman spectra were recorded in triplicate measurements from each solution. Raman bands at 1043 cm^{-1}, 870 cm^{-1}, and 565 cm^{-1} were selected as marker bands for aspirin, acetaminophen, and caffeine respectively. Peak areas of marker bands, after baseline subtraction, are plotted with respect to corresponding material's concentrations (Figure 7). All three solutes show linear relationships with respect to their concentrations.

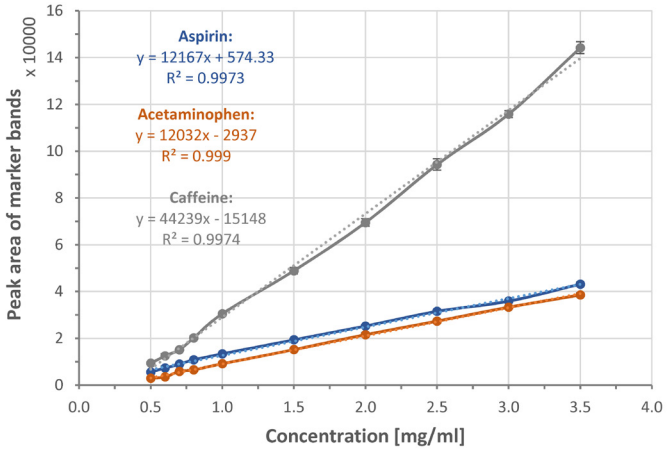


Figure 7: Calibration curves and linear trend lines (dotted lines) showing peak area of 1043 cm^{-1}, 870 cm^{-1}, and 565 cm^{-1}, marker bands of aspirin, acetaminophen, and caffeine respectively, plotted with respect to corresponding material's concentration. Each data point was averaged from 3 measurements. Error bars indicate standard deviations. Dotted lines are trend-lines. Their equations and R^2 values are shown in the plot.

6. Application of Calibration Curves

Raman spectroscopy can also be used to predict the concentrations of active pharmaceutical ingredients (APIs) in a dissolution study. In order to demonstrate this, we chose Excedrin® Extra Strength as a model system. Excedrin® Extra Strength contains three APIs: aspirin (250 mg), acetaminophen (250 mg), and caffeine (65 mg) per tablet. (6)

The conditions for dissolving the tablets were selected to mimic the administration of an oral dosage form. (7) Dissolution media was pH 2.4 HCl (~ 0.01 M) at 37 ºC, as this reflects gastric fluid conditions. Since aspirin is a drug with low water-solubility, we chose to include SLS, a surfactant to assist with wetting and dissolution of APIs. (8) SLS is a commonly used ingredient for this purpose in drug formulations. Excedrin® Extra Strength contains other inactive ingredients.

We dissolved each of four Excedrin® tablets in 100 ml of pH ~ 2.3 HCl at 37 °C overnight. The expected concentrations were ~ 2.50 mg/ml of aspirin and acetaminophen, and ~ 0.65 mg/ml of caffeine upon complete dissolution. It should be noted that the tablet did not completely dissolve. It partially dissolved, leaving a fine white precipitate at the bottom. We assume these residuals are inactive ingredients such as cellulose because we established that aspirin, acetaminophen and caffeine solubilities in HCl exceeds ~ 3 mg/ml in a separate study.

Raman spectra were recorded from 3 aliquots drawn from the dissolved portion. Raman bands at 1043 cm^{-1}, 870 cm^{-1}, and 565 cm^{-1} were selected as marker bands for aspirin, acetaminophen and caffeine respectively. Peak areas of these marker bands, after baseline subtraction, were measured. Any spectral contribution in this range from inactive ingredients were considered to be negligible, because the collective mass of APIs constitutes over 80% of the mass of the tablet. Dosages of APIs were predicted (Table 2) using linear calibration curves obtained above (Figure 7) and tablet weights.

	Aspirin	Acetaminophen	Caffeine
Tablet 1	240 mg	246 mg	66 mg
Tablet 2	239 mg	251 mg	67 mg
Tablet 3	241 mg	243 mg	66 mg
Tablet 4	242 mg	237 mg	67 mg

Table 2: Estimated API dosages per tablet of Excedrin® Extra Strength

Conclusion

We have demonstrated that Raman spectroscopy can be used to perform qualitative as well as quantitative analysis of materials. In this application note, we have shown several examples of how this can be applied for various systems. Raman spectra measured from a range of concentrations of a salt such as guanidine hydrochloride yielded a linear dependence of the signal. More complex solutions such as mixtures of ethanol and water were tested, where different degrees of hydrogen bonding depending on different ratios of individual component volumes lead to volume contraction. Depending on the concentration unit, the calibration curve can be linear or polynomial. Finally, calibration curves were built from aspirin, acetaminophen, and caffeine, and applied to pharmaceutical products such as Excedrin®, DayQuil™ and NyQuil™. We expect this to be of use in both dissolution testing studies as well as for studies in process and analytical systems.

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