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Noninvasive Identification of Materials inside USP Vials with Raman Spectroscopy and a Raman Spectral Library

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Abstract □ A commercial dispersive Raman spectrometer operating at 785 nm with a CCD detector was used to acquire spectra of USP reference materials inside amber USP vials. The laser and collection beams were directed through the bottom of the vials, resulting in a 60% loss of signal. The Raman shift was calibrated with a 4-acetamidophenol standard, and spectral response was corrected with a luminescent standard. After these corrections, the Raman spectra obtained inside the USP vial and on open powders differed by less than 5%. A spectral library of 309 reference materials was constructed, with spectral acquisition times ranging from 1 to 60 s. Of these, 8% had significant fluorescent background but observable Raman features, while 3% showed only fluorescence. A blind test of 26 unknowns revealed the accuracy of the library search to be 88–96%, depending on search algorithm, and 100% if operator discretion was permitted. The tolerance of the library search to degraded signal-to-noise ratio, resolution, and Raman shift accuracy were tested, and the search was very robust. The results demonstrate that Raman spectroscopy provides a rapid, noninvasive technique for compound identification.

Introduction

Vibrational spectroscopy in the form of Fourier transform infrared (FTIR) absorption has long been used for materials identification. The combination of a rich spectral fingerprint and extensive spectral libraries results in a reliable and straightforward method for qualitative analysis of solid and liquid pharmaceuticals. However, FTIR generally requires sample preparation, and mid-infrared (MIR) light is strongly absorbed by most sample containers and fiber optics. So MIR spectroscopy is generally unsuitable for noninvasive observation of samples in glass or plastic containers and is difficult to interface with a remote location via fiber optics. The extension of MIR to noninvasive sampling would be very valuable for on-line or at-line process monitoring, but this prospect appears difficult except for special cases.

Near-infrared (NIR) absorption uses light in the 1−3 μm wavelength range and is compatible with fiber optics and glass containers. Remote and/or noninvasive sampling has been a major driving force for the development of NIR, particularly for quality control and process monitoring. Unfortunately, NIR absorption is based on combinations and overtones of mainly C−H stretches, and NIR spectra are not as information-rich as MIR spectra, which are based on fundamentals of a wider variety of molecular vibrations. Furthermore, NIR analyses often require multivariate calibration, which can be complicated by variations in water content or humidity. While NIR absorption is attractive from the standpoint of cost and sampling flexibility, the resulting spectra are not as specific nor as accurate as those from MIR spectrometers.

Although Raman spectroscopy has only recently been developed for analytical purposes, it has existed for more than half a century as a vibrational technique which provides spectral information similar to and often comple-

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mentary to MIR spectroscopy. Like MIR, Raman is based on fundamental vibrations and provides detailed spectral "fingerprints", but like NIR, Raman uses light which is compatible with fiber optics and many sample containers.[1,2] The selection rules for MIR and Raman are different, although in many cases the same molecular vibrations are observed. Raman requires a polarizability change while MIR or NIR requires a dipole moment change; hence, Raman is preferred for symmetric vibrations present in aromatic molecules, −S−S− bonds, C=C bonds, etc. Of value in pharmaceutical analysis is the relative strength of Raman scattering of aromatic drug substances compared to nonaromatic excipients, providing some selectivity for the drug. Raman spectra may be obtained noninvasively from solids and liquids inside vials or blister packs. This attractive combination of sampling and information content has not been exploited in the past for routine analysis because Raman used to be a complex and difficult technique, plagued by low sensitivity and fluorescence interference. These impediments have largely been eliminated by modern technology, notably NIR excitation (not to be confused with NIR absorption), CCD (charged coupled device) detectors, FT-Raman, and inexpensive computers.[2,3] Reliable, integrated Raman spectrometers are now available commercially[4] and are rapidly gaining acceptance as useful instruments for analytical chemistry. Applications of modern Raman spectroscopy to the analysis of pharmaceutical solids have been reviewed.[5]

Libraries of FT-IR spectra are extensive,[6,7] in both printed and electronic form, and new techniques continue to be developed. Several printed compendia of Raman spectra and characteristic frequencies are available,[7−10] and electronic versions are beginning to emerge. Specialized libraries for particular problems have been reported, including one for identification of urinary calculi[11] and for identifying spots on TLC plates.[12] A >5000 member FT-Raman library is available commercially in electronic form.[13]

The current work was undertaken to evaluate dispersive Raman spectroscopy with a 785 nm laser for identification of solid pharmaceuticals in amber vials. A 309-member spectral library was constructed from USP reference materials in USP vials, with the Raman light excited and collected through the vial bottom. Commercially available software (GRAMS/32, Galactic, Inc., Salem, New Hampshire) was used to search the test library and identify a group of blind "unknowns". The sensitivity of the search process to experimental parameters such as signal/noise, frequency error, and resolution was examined.

Experimental Section

The Raman spectrometer was a prototype version of the Chromex Raman 2000 with a 2050 sample chamber. Light from a Spectra Diode Labs SDL 8530 laser (785 nm) passed through a dielectric band-pass filter and then was focused through the bottom of standard USP amber vials. Backscattered light was collected by the same lens, passed through a Kaiser Notch Plus holographic laser rejection filter, and imaged onto the entrance slit of a Chromex 250 mm focal length imaging spectrograph. The detector was a EEV 15−11 (deep depletion) CCD in a Photometrics 270 controller. Unless noted otherwise, spectral conditions were as follows: laser power at sample, 50 mW on an approximately 100 μm diameter spot; 600 line/mm (1 μm optimized) grating; 50 μm slit width (resolution based on slit width = 3.4 cm^{-1} at 1500 cm^{-1}); spectral range 210−2060 cm^{-1}. Laser focus was adjusted for maximum signal from calcium ascorbate and then not readjusted for unknowns. Integration times varied from 0.1 to 60 s. Approximately 30 mW of laser light was transmitted to the sample through the USP vial, and there is a possibility of sample degradation for susceptible materials. None was observed in the current work, but prolonged integration times were not considered or required.

Raman shift was calibrated with naphthalene or acetamidophenol Raman shift standards (ASTM E 1848) and a quadratic fit of observed to standard values. Calibration with 10 peak frequencies for acetamidophenol between 329 and 1648 cm^{-1} yielded the most reproducible Raman shift values. Relative intensity was calibrated with a luminescent standard as described previously.[14] A sample of Kopp 2412 glass was placed inside an empty USP vial and its luminescent spectrum was obtained. The polynomial coefficients for the luminescence curve were those reported previously.[14] The reproducibility of these calibrations is discussed in the Results section.

A total of 309 USP standard reference materials were borrowed from the FDA drug analysis lab in St. Louis. GRAMS/32 Galactic, Inc. version 4.11 level II, and the "IR Search" add-on (version 3.14 initially, 3.18 finally) were used to construct and search the library. Library creation parameters were as follows: 1000 data points, 250−2000 cm^{-1}, 16 bit precision, *X*-axis in Raman shift, *Y*-axis in arbitrary units. Before spectra were added to the library, a two-point baseline chosen by Grams (with "baseline.ab") was subtracted from the spectrum after intensity calibration. The default points chosen by Grams were used, to avoid operator intervention and possible biasing. Of the 309 samples, 25 (8%) exhibited a relatively large fluorescent background, but still had observable Raman features, and 8 more (2.5%) showed only fluorescence. These samples were included in the library without discrimination. Before each day's acquisition of library spectra, instrument operation was verified by checking that frequency and relative intensity were within the limits described below. Library search algorithms are discussed in the results section.

Results

Dispersive/CCD spectrometers sample a particular Raman shift range, depending on grating selection, grating position, CCD size, etc. Figure 1 shows three spectra of calcium ascorbate, obtained with 300, 600, and 1200 line/mm gratings. Since there is a fixed number of CCD pixels along the wavelength axis (1024), there is an inherent tradeoff of resolution and spectral coverage. Thus the 1200 line grating yields high resolution (1.7 cm^{-1} for a 50 μm slit), but lower spectral coverage (~1000 cm^{-1}) than the 600 line grating (3.4 cm^{-1} and 2000 cm^{-1}). The library search software does not require that the spectral range of the unknown spectrum match that of the library, but it does require that all library spectra have the same spectral range. Because of the lack of Raman features between 1800 and 2800 cm^{-1} (for most samples), and because of the lower sensitivity of CCD's to the C−H stretch region (>2800 cm^{-1}), the 600 line grating was chosen to construct the library. This choice is a compromise of resolution and spectral coverage, at the cost of spectral information above 2000 cm^{-1}.

Figure 2 shows four spectra of pharmaceuticals obtained from solids inside USP vials. The great majority of the 309 USP samples exhibited spectra similar to the top two in Figure 2, with well-defined Raman features and integration times of 1−60 s. Ethylcellulose is an example of observable Raman features superimposed on a large fluorescence background. Approximately 8% of the spectra had a similar appearance. About 3% of the samples (8 out of 309) showed only fluorescence, such as dichlorophenylindolinone. Such samples are not amenable to Raman-based library searches, but even relatively small Raman features superimposed on fluorescence (such as for ethylcellulose) permit reliable qualitative identification. The fluorescence interference may be inherent in the molecule of interest, or it can be caused by fluorescent impurities. In general, fluorescence is decreased by operating at longer laser wavelengths, so a 785 nm system will exhibit fewer fluorescent samples than a 515 nm spectrometer but more than FT-Raman operating at 1064 nm.

The effect of sampling through an amber vial is illustrated in Figure 3 for acetamidophenol as an open

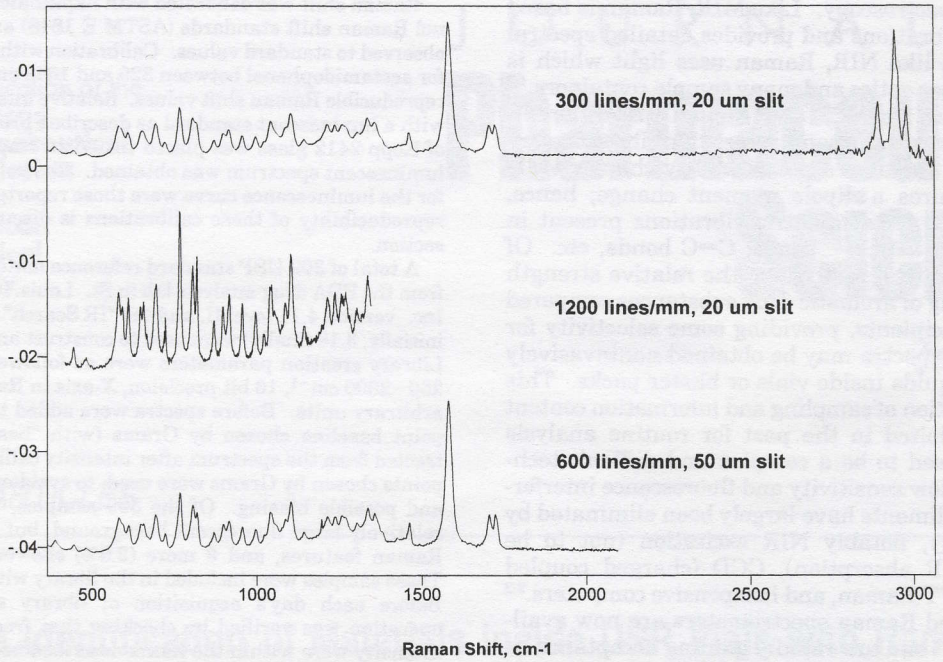

Figure 1—Raman spectra of calcium ascorbate acquired through the bottom of a standard USP vial. Three gratings were employed, and each spectrum is corrected for instrumental response; 52 mW (785 nm) light entering the vial and the integration time of 10 s in all cases.

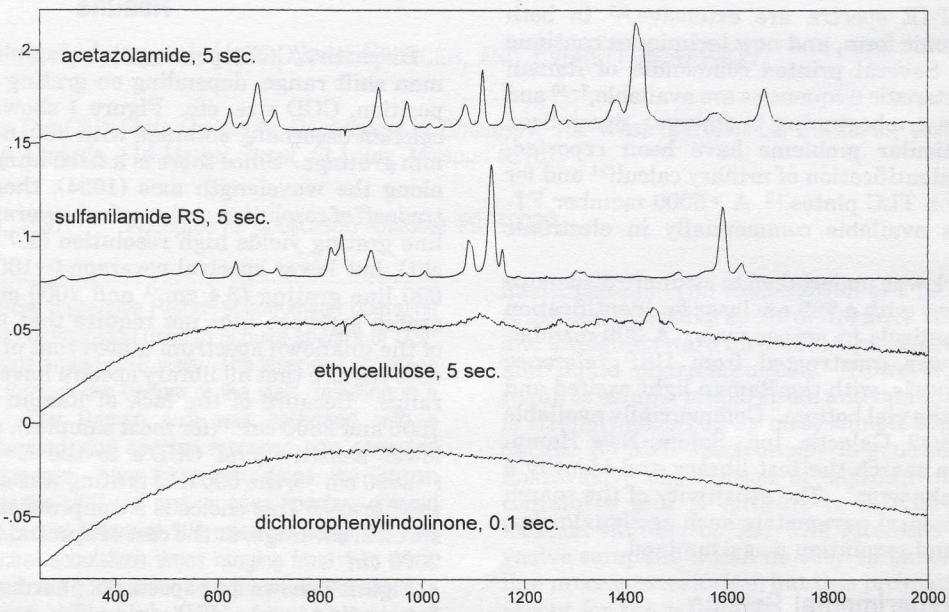

Figure 2—Raman spectra of USP reference materials inside USP vials: 600 line/mm grating, 50 μm slit width, 52 mW light entering vial. Compound names and integration times are as shown, and all spectra were corrected for instrument response.

powder and inside a USP vial. The spectrum of the open powder was corrected for relative intensity with an open piece of Kopp 2412 glass, while the vial spectrum was corrected with the Kopp glass inside the vial. For both sample and glass spectra, the focus was adjusted for maximum intensity, and the vial spectrum was subtracted as appropriate. The uncorrected spectra of both acetamidophenol and Kopp glass showed that the amber vial attenuated the signal to 41% of its value for the open sample, implying that the amber glass transmits about 64% each of the laser and Raman light. Since losses by vial transmission apply to both the sample and the Kopp glass intensity standard, they are corrected by the intensity calibration. The corrected spectra of acetamidophenol in Figure 3 differ in intensity by 5%. When the spectra are subtracted after adjusting for this difference, the maximum residual is 4%, and there is no trend across the Raman shift range observed. So both relative peak intensities and total Raman signal are corrected for vial transmission using the luminescent standard. Reproducibility of Raman frequencies and intensities was evaluated by obtaining spectra of calcium ascorbate in a USP vial over a several month period. The GRAMS peak-picking algorithm, which uses a center-of-gravity approach, was used to determine peak frequencies. For eleven Raman peak frequencies of calcium ascorbate, the standard deviations for six replicates on six different days ranged from 0.18 to 0.79 cm^{-1}, with all but one below 0.50 cm^{-1}. Twenty-three determinations of two calcium ascorbate frequencies over a 2 month period yielded standard deviation of 0.14 cm^{-1} for the 767.3 cm^{-1} band and 0.22 cm^{-1} for the 1582.7 cm^{-1} band. For fifteen replicates performed in one session but with random vial rotation between runs, three peaks showed standard deviations of 0.18–0.20 cm^{-1}. The raw intensities of Raman

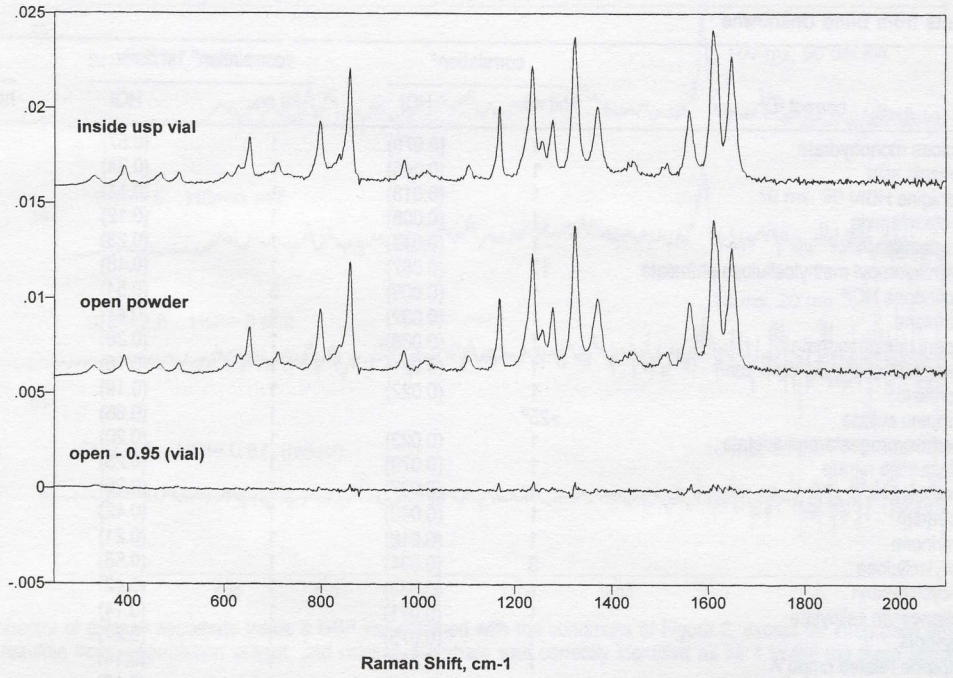

Figure 3—Raman spectra of 4-acetamidophenol obtained inside a USP vial and as an open powder, both corrected for instrument response. Conditions were the same as for Figure 2. The lower spectrum is the difference after multiplying the vial spectrum by 0.95. The same intensity scale was used in all cases.

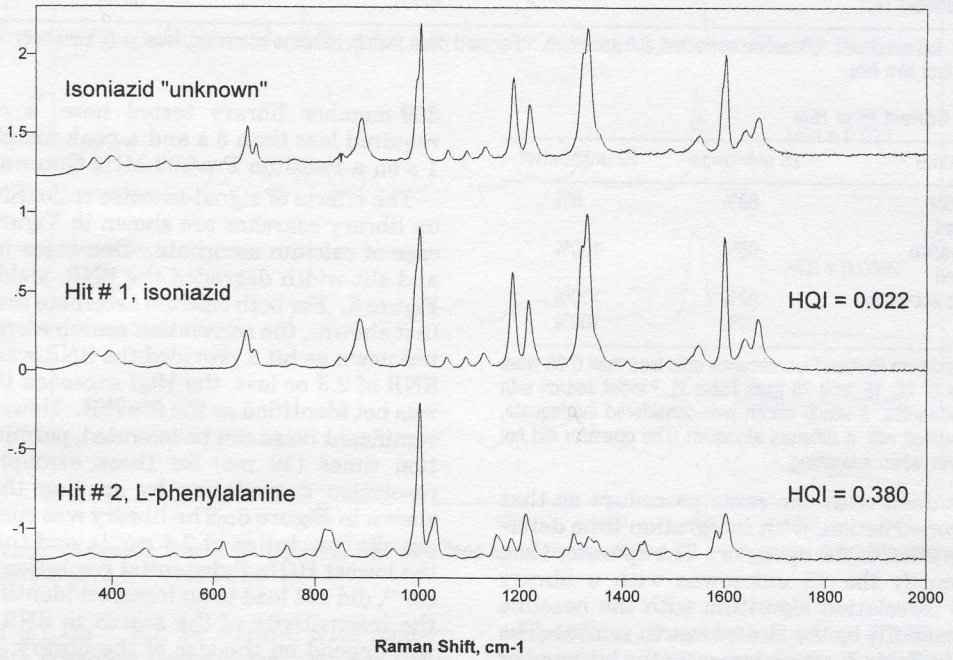

Figure 4—Example of a library search using the full spectrum/correlation search with baseline subtraction. "Hit 1" refers to the best match of the upper ("unknown") spectrum to 309 spectra in the library. HQI is the hit quality index, as discussed in the text.

bands varied approximately ±15% from day to day over a 2 month period, and about 5% for 15 runs on the same day with the vial rotated randomly between runs. The corrected peak intensity for the 1582 cm^{-1} calcium ascorbate band was 0.0116 ± 0.0009 (8%), and for the 767 cm^{-1} band, 0.00543 ± 0.00044 (8%) for 23 spectra over 2 months. The 767/1582 peak intensity ratio for these corrected spectra was 0.466 ± 0.011 (2.4%).

A typical library search result is shown in Figure 4, for a sample of isoniazid which was run separately from the library spectrum and whose identity was unknown to the operator. "Hit 1" is the first choice by the library search software from the 309-member USP library, "hit 2" is the second choice. Selection in this case was based on a "correlation" search, which searches for the library spectrum having the minimum difference for all Raman shifts, compared to the sample spectrum. Before this difference is calculated, both known and library spectra are normalized and mean-centered, which reduces differences in intensity and baseline offset. The correlation search is similar to, but generally more successful than the widely used "Euclidean distance" algorithm.[15] The "hit quality index" (HQI) ranges from zero for a perfect match to 1.0 for uncorrelated spectra. The low value of HQI for hit 1 in Figure 4 and the large increase in HQI for hit 2 adds confidence that hit 1 is correct.

Library accuracy was tested with a blind study of 26 unknowns chosen at random from the 309 USP standards.

Table 1—Search Results from Blind Unknowns

unknown	correct ID	correlation[a] hit no.	correlation[a] HQI	correlation[b] 1st deriv hit no.	correlation[b] 1st deriv HQI	peak match[c] hit no.	peak match[c] HQI
1	lactose monohydrate	1	(0.079)	1	(0.57)	1	(65.6)
2	benzoic acid	1	(0.045)	1	(0.24)	1	(99.2)
3	clonidine HCl	1	(0.018)	1	(0.11)	1	(85.5)
4	acetazolamide	1	(0.008)	1	(0.12)	1	(73.0)
5	guaifenesin	1	(0.042)	1	(0.23)	1	(50.8)
6	hydroxypropyl methylcellulose phthalate	17	(0.067)	1	(0.48)	1	(29.7)
7	molindone HCl[b]	1	(0.003)	5	(0.54)	4	(26.6)
8	naloxone	1	(0.032)	1	(0.44)	1	(58.6)
9	piperazine phosphate	1	(0.068)	1	(0.26)	1	(59.0)
10	beclomethasone dipropionate	1	(0.021)	1	(0.18)	1	(54.7)
11	isoniazid	1	(0.022)	1	(0.19)	1	(70.3)
12	atropine sulfate	>25[d]		1	(0.66)	1	(26.6)
13	medroxyprogesterone acetate	1	(0.023)	1	(0.20)	1	(65.2)
14	pilocarpine nitrate	1	(0.070)	1	(0.25)	1	(47.3)
15	mebrofenin	1	(0.037)	1	(0.25)	1	(51.09)
16	estradiol	1	(0.040)	1	(0.42)	1	(48.4)
17	amrinone	1	(0.016)	1	(0.21)	1	(70.7)
18	ethylcellulose	3	(0.034)	1	(0.55)	2	(26.1)
19	β-cyclodextrin	1	(0.083)	1	(0.42)	1	(55.9)
20	magnesium salicylate	1	(0.011)	1	(0.14)	1	(84.0)
21	lisinopril	1	(0.075)	1	(0.27)	1	(77.3)
22	glipizide related cmpd A	1	(0.088)	1	(0.14)	1	(77.3)
23	sulfanilamide RS	1	(0.015)	1	(0.13)	1	(75.0)
24	cisplatin	1	(0.038)	1	(0.26)	1	(44.1)
25	*p*-toluenesulfonamide	1	(0.16)	1	(0.15)	1	(31.3)
26	carteolol HCl	2	(0.23)	1	(0.14)	1	(53.9)

[a] Baseline corrected, full spectrum. [b] Baseline corrected, full spectrum. [c] Forward peak match, baseline corrected, level = 0, sensitivity = 10, standard. [d] Correct compound was not in first two hits.

Table 2—Percentage Correct First Hits

search procedure	26 unknowns	22 unknowns[a]
full spectrum correlation, baseline subtracted	88%	6%
correlation, first derivative, baseline subtracted	96%	100%
peak match, baseline subtracted	92%	100%
operator discretion[b]	100%	100%

[a] Unknowns with maximum Raman/Fluorescence ratio less than 0.30 were not included. (numbers 6, 12, 18, and 26 from Table 2). [b] Initial search with correlation/baseline subtracted. If visual match was considered inadequate, the spectra was researched with a different algorithm. The operator did not know the correct answer when searching.

Spectra were obtained with the same procedure as that used for library construction, with integration time determined at the discretion of the operator. The operator then attempted to identify the 26 unknowns with a library search using the correlation algorithm with the baseline subtracted automatically by the Grams search routine. The results are listed in Table 1, which indicates the hit number for the correct identification and the hit quality index. For both correlation searches, a HQI of 0 is perfect and 1 is uncorrelated, while for the peak match, a perfect match has a HQI of 100. Of the 26 unknowns, the library correctly identified the unknown as hit 1 for 22. All four misidentifications had high fluorescence, similar to the ethylcellulose spectrum in Figure 2. Before the list was unblinded, the author noted the poor visual match for these four (unknowns 6, 12, 18, and 26), and searched again with the "correlation, first derivative" algorithm, with and without baseline subtraction. Once the operator was satisfied with the visual match, all four were correctly identified as hit 1. Table 2 shows results for searches with different algorithms, with and without the four unknowns exhibiting high fluorescence. "Peak match" is a much faster search process which compares only peak positions and heights and is often used as an initial search. For the 309-member library tested here, a correlation search required less than 3 s and a peak match search less than 1 s on a Pentium Pro/200 MHz Gateway PC.

The effects of signal-to-noise ratio (SNR) and resolution on library searches are shown in Figures 5 and 6 for the case of calcium ascorbate. Decreases in integration time and slit width degraded the SNR, yielding the spectra of Figure 5. For both calcium ascorbate and acetamidophenol (not shown), the correlation search correctly identified the unknown as hit 1 provided the SNR was 3 or greater. For SNR of 2.5 or less, the HQI exceeded 0.6 and the sample was not identified as the first hit. However, it is clear that significant noise can be tolerated, permitting short integration times (10 ms) for these examples. The effect of resolution degradation by opening the entrance slit is shown in Figure 6. The library was constructed with a 50 μm slit (resolution of 3.4 cm^{-1}), and this slit width yields the lowest HQI. Substantial resolution degradation, to 34 cm^{-1}, did not lead to an incorrect identification. Of course, the insensitivity of the search to SNR and to resolution will depend on the size of the library, and the process is likely to be less forgiving with a larger library. Furthermore, a more sensitive spectrometer will yield a higher SNR for a given measurement time, and this higher sensitivity should improve the accuracy of the search.

In part because calibration procedures used in different labs have not been well-standardized, there is some variation in Raman shift values reported in the literature. These variations are generally 1–2 cm^{-1}, but can be as large as 5 cm^{-1}. The question arises of the sensitivity of library searches to such variations in observed peak frequencies, particularly since a given library may be used with different instrument types. To investigate this issue, the Raman shift axes of spectra of isoniazid and cisplatin were intentionally offset before searching. As shown in Table 3, the correlation search was fairly insensitive to Raman shift offsets up to 5 cm^{-1}. The peak match failed with smaller offsets, of 2 or 3 cm^{-1}. It is likely that Raman

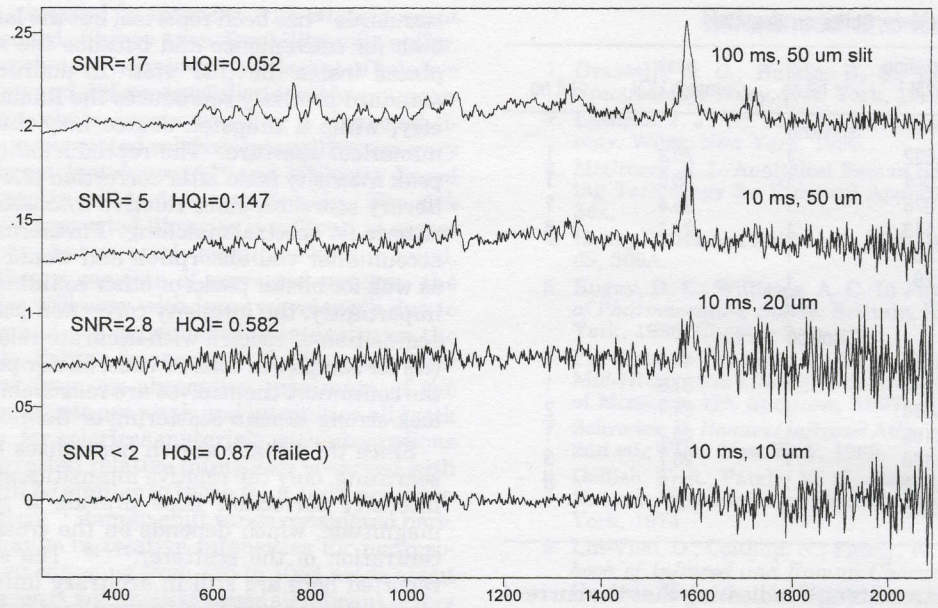

Figure 5—Corrected spectra of calcium ascorbate inside a USP vial obtained with the conditions of Figure 2, except for integration times and slit widths shown at the right. The HQI resulted from a correlation search, and calcium ascorbate was correctly identified as hit 1 in the top three spectra.

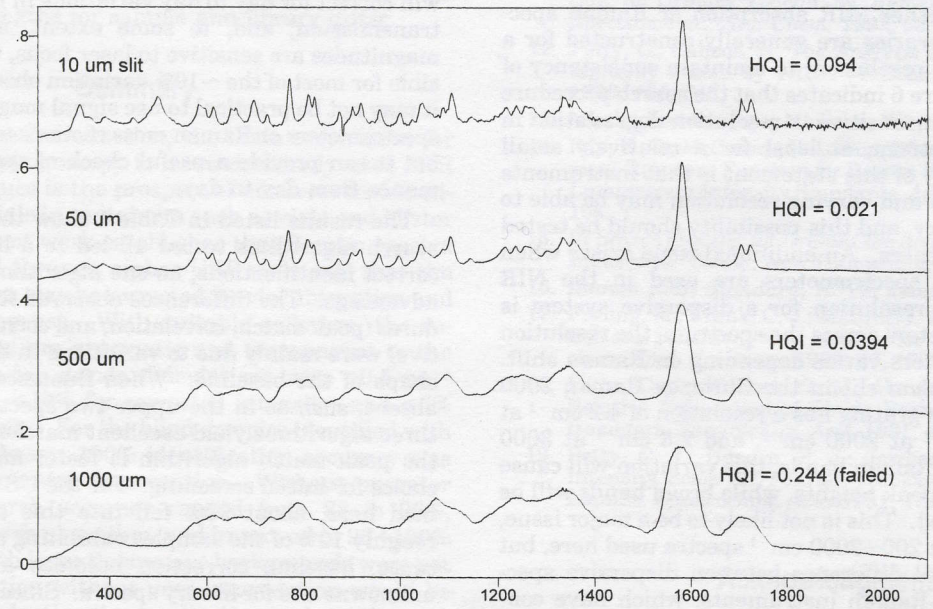

Figure 6—Corrected calcium ascorbate spectra (in USP vial) with conditions as in Figures 2 and 10 s integration time. Slit widths correspond to spectral resolutions (top to bottom) of <2, 3.4, 34, and 68 cm^{-1}.

shift accuracy of ± 1 cm^{-1} will be routine with modern instruments, and a standard deviation of <0.5 cm^{-1} was observed here for the 1582 cm^{-1} band of calcium ascorbate. So frequency inaccuracy should not be an issue if reasonable calibration procedures are followed.

Discussion

As noted in the results section, a dispersive multichannel spectrometer faces a tradeoff of spectral coverage and resolution, and the choice here was to use the 250–2000 cm^{-1} Raman shift range for the library. This range includes the "fingerprint" and "group frequency" regions and proved to be adequate for the unknowns and library size investigated. Since the C–H stretch region was not included, the question arises of how compound identification is affected by the absence of the C–H stretch frequencies. An advantage of FT-Raman is full spectral coverage regardless of resolution, so an FT library would be more sensitive to differences in C–H stretches. Since an analogous library with full spectral coverage is not currently available, the question of the cost of ignoring the C–H stretch region for the 309 compounds studied remains open.

Frequency accuracy is an important requirement for library searching and for spectral subtraction of spectral contributions from excipients or other materials. Fourier transform Raman instruments enjoy excellent frequency precision, but dispersive spectrometers do exhibit drift and jitter, sometimes as large as a few wavenumbers. The frequency precision observed for the current system of ± 0.50 cm or less was achieved by daily calibration with a Raman shift standard. Since the library searches were able to tolerate 2 cm^{-1} or more of frequency offset, the observed frequency precision should be more than adequate. In fact, ± 0.50 cm^{-1} is approximately equal to the standard deviation for a comparison of five FT-Raman and

Table 3—Effect of Frequency Shifts on Searches

frequency offset, cm^{-1}	correlation HQI	hit no.	peak match HQI	hit no.
		Isoniazid		
0	0.022	1	70.3	1
1	0.046	1	38.6	1
2	0.076	1	25.4	1
3	0.113	1	21.1	8
5	0.20	1		
7	0.29	1		
10	—	>10		
		Cisplatin		
0	0.027	1	44.1	1
1	0.054	1	31.6	1
2	0.078	1	21.1	2
3	0.106	1	29.7	1
4	0.138	1	20.7	3
5	0.173	1	20.3	8
10	0.348	1	—	

two dispersive spectrometers,[16] indicating that the current dispersive/CCD system has acceptable frequency accuracy.

Although variations in spectral resolution should not affect relative Raman band areas, they will affect relative peak heights, in either MIR absorption or Raman spectroscopy. FTIR libraries are generally constructed for a particular spectral resolution, to maintain consistency of peak heights. Figure 6 indicates that the search procedure used here is quite insensitive to resolution degradation in the unknown spectrum, at least for a relatively small library. A corollary of this statement is that instruments of different designs and varying resolution may be able to use the same library, and this possibility should be tested as library use increases. An additional issue arises when dispersive Raman spectrometers are used in the NIR region. Since the resolution for a dispersive system is constant in nanometers across the spectrum, the resolution in inverse centimeters varies depending on Raman shift. For example, a 50 μm slit in the Chromex Raman 2000 with a 600 line/mm grating has a resolution of 4.2 cm^{-1} at 200 cm^{-1}, 3.1 cm^{-1} at 2000 cm^{-1}, and 2.6 cm^{-1} at 3000 cm^{-1}. For narrow Raman bands, this variation will cause some distortion of peak heights, while broad bands will be only weakly affected. This is not likely to be a major issue, particularly for the 200–2000 cm^{-1} spectra used here, but it is a fundamental difference between dispersive spectrometers and FT-Raman instruments, which have constant resolution in inverse centimeters across the Raman spectrum.

Raman intensity calibration, both relative and absolute, is a much more complex issue than frequency calibration. MIR and NIR methods are based on a ratio of incident to transmitted light, while Raman generally involves only observation of scattered light. Since a MIR or NIR spectrometer measures the incident intensity, it corrects for instrumental response as a function of wavelength. Any variations in response with wavelength or time are compensated when the absorbance is calculated from incident and transmitted signals. Since Raman spectroscopy is generally a "single beam" technique without a reference intensity, Raman intensity is subject to rather large variations from detector response, focusing, alignment, laser power, transmission, etc. Such variations affect both the magnitude of the observed Raman signal and the relative intensities of peaks within a spectrum. Most of the Raman spectra in the literature are uncorrected for these possibly major variations in instrument response.

Response correction of dispersive Raman spectrometers with standard tungsten sources[17] and with luminescent standards[14] has been reported, but the latter was used here both for convenience and because the standard could be placed inside the USP vial. In addition, a luminescent standard precisely reproduces the Raman sampling geometry, while a tungsten source may differ in position or numerical aperture. The reproducibility of the 767/1582 peak intensity ratio after correction (2.4%) is important to library searches, since relative intensities are among the criteria for spectral matching. Furthermore, the correction accounts for vial absorption and would presumably work as well for blister packs or other containers. Perhaps most importantly, the intensity correction permits noninvasive acquisition of spectra with accurate relative peak intensities for samples in colored vials, blister packs, etc., provided the containers themselves are reasonably transparent and lack strong Raman scattering or fluorescence.

Since the library search normalizes the spectra before searching, only the relative intensities are important. The intensity correction does provide a meaningful signal magnitude, which depends on the cross section and concentration of the scatterer.[17–19] The signal magnitudes reported here are still in arbitrary units, since the Kopp glass emission was itself normalized before its polynomial was determined.[14] However, the signal magnitude does indicate scattering strength relative to the standard and will correct for day to day variations in laser power, optics transmission, and, to some extent, alignment. These magnitudes are sensitive to laser focus, which was responsible for most of the ~10% variation observed. At present, it may not be practical to use signal magnitude to compare spectrometers or Raman cross sections with high accuracy, but it can provide a useful check of spectrometer performance from day to day.

The results listed in Table 1 show that while the three search algorithms tested all led to a high percentage of correct identifications, no one algorithm exhibited a clear advantage. The differences observed for the three procedures (peak match, correlation, and correlation/first derivative) were mainly due to variations in the magnitude and shape of the baseline. When fluorescence was small or absent, such as in the upper two spectra of Figure 2, all three algorithms yield excellent matches. For such cases, the peak match algorithm is faster and is a reasonable choice for initial screening. For the USP standards examined here, about 90% fell into this category. For the roughly 10% of the samples exhibiting significant fluorescence, baseline correction becomes an issue, both for unknowns and for library spectra. Since the slowly varying baseline rarely contains any vibrational information, the safest approach is to use the correlation/first derivative algorithm. However, this approach is sensitive to noise and may fail for spectra containing spikes or discontinuities. The Grams software includes a linear baseline subtraction (srchbase.abh) based on the GIFTS algorithm, which results in a spectrum with all-positive points after subtracting a line whose slope and intercept are determined by the algorithm. The automatic two-point baseline correction used here is another Grams option, as are various procedures based on polynomial fits to the baseline or Fourier smoothing.

A major issue in any application of library searching is the transferability of a library to instruments of a different manufacturer or design. For a given laser wavelength, libraries of corrected spectra should be transferable, particularly if the resolution is kept constant. The intensity correlation compensates for instrument response and absorption by the sample container, so relative intensities should be reproducible for different instruments. Figure 6 shows that the search tolerates significant resolution changes, a fact which should improve transferability. If

the library is not corrected for intensity with a luminescent or tungsten standard, library transferability will suffer. The search approach used here is fairly tolerant of relative intensity variation and did successfully identify an uncorrected calcium ascorbate spectrum using a corrected library. However, uncorrected relative intensities can vary severely for different instruments,[14] and a library based on uncorrected spectra will be less transferable. The risk entailed in using uncorrected libraries is difficult to assess in a general manner but certainly should be kept in mind.

Even if the library consists of corrected spectra, the relative intensities will vary with laser wavelength due to resonance effects and the dependence of intensity on the fourth power of the Raman frequency (ν_r^4). When the laser wavelength is far from an absorption maximum of the sample, resonance effects are weak and intensities all track ν_r^4. For example, for colorless materials with absorptions in the UV, the corrected relative intensities observed with a 785 nm laser will be similar to those for a 1064 nm laser. For the 250–2000 cm^{-1} Raman shift range considered here, the maximum change in relative intensities for nonresonant samples when changing from 785 to 1064 nm is about 20%. This difference is not likely to cause significant errors for library searches conducted with 785 or 1064 nm spectrometers. Nevertheless, resonance or preresonance effects can be present, and care should be exercised when the laser wavelengths for sample and library differ.

Summary

As noted in the Introduction, the main motivation for adding Raman spectroscopy to more commonly used MIR and NIR techniques is the prospect of noninvasive identification of materials in containers such as vials and blister packs. The current work establishes that Raman spectra may be obtained through amber USP vials and that the relative intensities may be corrected for vial absorption and instrumental response. With suitable software, the required corrections are automatic and transparent to the operator. Day to day standard deviations were <0.5 cm^{-1} for Raman shift, <10% for corrected intensity, and <3% for relative intensity. For the blind samples identified with a 309-member library, 100% identification accuracy was achieved with operator intervention. Without operator intervention, search accuracy ranged from 88 to 96%, depending on search algorithm, and improved to 96–100% when samples with large fluorescent backgrounds were not included. Operation without user intervention would be important for production line applications, where the user may be unfamiliar with spectroscopy. For a large number of analyses where fluorescence can be avoided, an unattended library search can yield an accurate materials identification, while retaining the advantages of noninvasive operation, no sample preparation, and rapid analysis time.

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