Advantage of handheld Raman spectrometer with 1064 nm excitation in pharmaceutical raw material identification

Taro Nogami[*] **and Fumihito Muta**[*]

1.　Introduction

Quality control is a top priority for the pharmaceutical industry. RMID (raw material identification) is an important part of quality control. Since health authorities of many countries including Japan joined PIC/S[(1)] recently, quick and reliable RMID[(2)] is becoming more important.

In the field of RMID, IR (infrared) and NIR (near infrared) spectrometry were used earlier than Raman spectroscopy. However, these analytical methods have limitations in the types of materials they can analyze. Raman spectroscopy can solve the many limitations or problems that were encountered in the past and is now widely recognized as a useful method for material identification.

One of the important advantages of Raman spectroscopy over IR and NIR spectroscopy is the fact that water does not cause interference, allowing measurement of moist samples and even aqueous solutions.

Another advantage is that the Raman spectroscopy enables direct material identification through a glass bottle or a transparent bag, thus minimizing the risk of contamination. In addition, Raman spectroscopy has an advantage over NIR spectroscopy regarding the ease of material identification. This advantage is due to the abundant, yet sharp and well resolved, spectral peaks in the Raman spectra. These sharp and abundant peaks in Raman allow for material identification without the need to build time consuming chemometric models as is required in NIR spectroscopy. Finally, inorganic materials are normally easier to analyze by Raman spectroscopy than by NIR spectroscopy which shows very weak peaks for inorganic materials.

2.　Handheld Raman spectrometer

Figure 1 shows Rigaku's new handheld Raman spectrometer, Progeny. It has several important features for pharmaceutical applications. First of all, it utilizes 1064 nm excitation wavelength. Existing handheld Raman spectrometers traditionally use shorter excitation wavelengths, such as 532 nm and 785 nm. One of the major limitations of existing handheld Raman spectrometers using shorter wavelengths is fluorescence interference. For some types of materials, high fluorescence background creates detector saturation or causes incorrect material identification. For instance, pigments, many colored excipients, animal/

plant origin materials, cell culture media and many cellulosic materials are difficult to analyze by the shorter wavelengths. However, Progeny, which has 1064 nm laser, can analyze these materials. Highly fluorescent glass bottles are also troublesome for the shorter wavelength excitation. This problem also can be minimized by the 1064 nm excitation.

The second advantage of the Progeny is an adjustable nose cone. The nose cone, which contacts the sample, has a feature as shown in Fig. 2. Since there is a position scale and click stop for the nose cone, the position can be selected easily depending on sample conditions.

Figure 3[(3)] shows an analysis of liquid contained in a glass bottle taken through the glass. By shifting the nose

Fig. 1.　Handheld Raman spectrometer Progeny.

Fig. 2.　Adjustable focal point nose cone.

* Portable Analyzer Division, Rigaku Corporation.

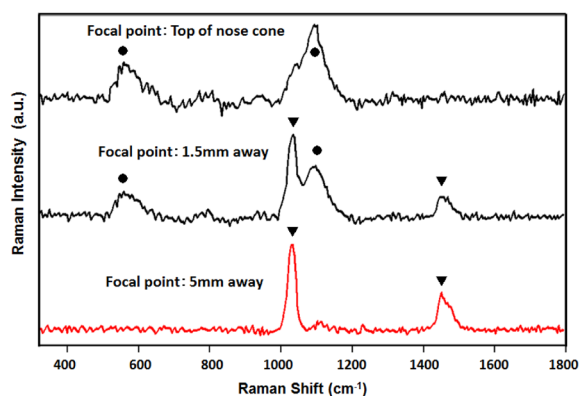**Fig. 3.** Analysis of liquid contained in a glass bottle.

Fig. 4. Spectra of methanol in the glass bottle with different focal point settings.

cone position towards the chassis of Progeny, the position of the focal point is shifted away from the nose cone, and placed inside of the glass bottle. Figure 4[3] shows spectra of methanol in the glass bottle with different focal point settings. When the focal point is at the top of the nose cone, mainly the surface of the glass is analyzed. Stretching vibration peaks of Si−O at $1092\,cm^{-1}$ and Si−O−Si at $550\,cm^{-1}$ are observed. Both peaks are Raman peaks of the glass, and are marked by "●" in Fig. 4. However, by shifting the focal point further from the nose cone, these peaks become lower, and the stretching vibration peak of C−O at $1036\,cm^{-1}$ and deformation vibration peak of CH_3 at $1458\,cm^{-1}$ become higher. They are Raman peaks of methanol, and are marked by "▼". By setting the focal point $5\,mm$ away, the peaks of the glass can be eliminated. This focal point adjustment capability, removes the need to open bottles or packages before making a measurement. This function enables RMID in a warehouse, a receiving dock and any location, without sampling and without a contamination risk.

The smart-phone inspired interface provides intuitive operation and decreases the learning time.

A user with administrator access can adapt Progeny into any existing workflow by optimizing analysis conditions and libraries. Operators simply use the
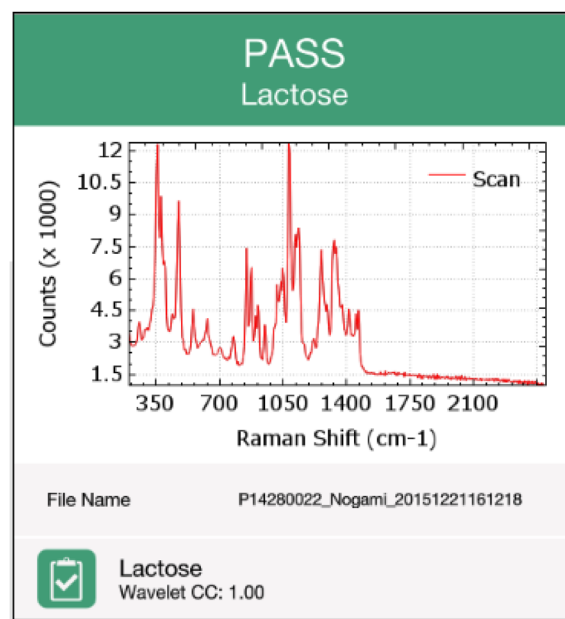

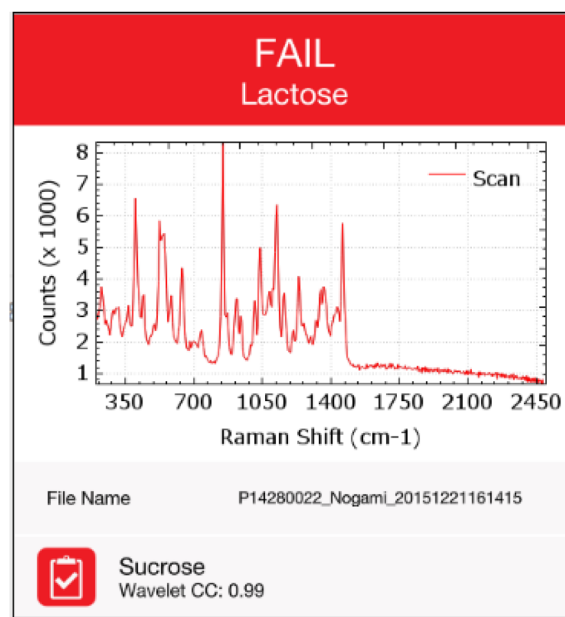**Fig. 5.** Screen of positive verification result.

Fig. 6. Screen of negative verification result.

analysis conditions prepared by the administrator, and obtain material identification results easily and quickly.

Figures 5 and 6 are examples of material verification results. Figure 5 shows the screen of positive verification result (PASS) of lactose. It proves the tested material is really lactose. Figure 6 shows negative verification result (FAIL) of lactose. It means the tested material is not lactose. The bottom part of the screen shows that the material is actually sucrose.

3. Advantage of 1064 nm excitation for complex or difficult samples
3.1. Cellulosic materials
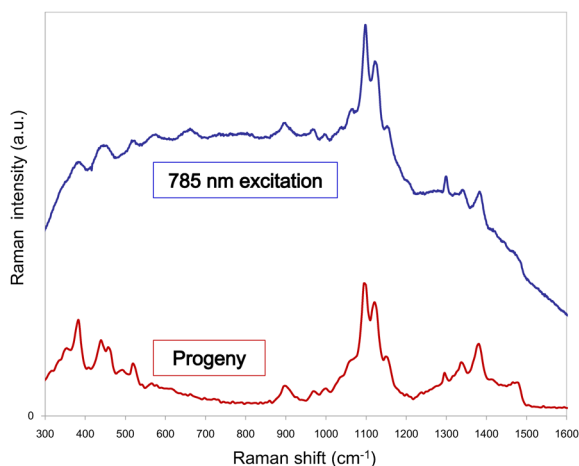Cellulosic materials are important excipients and
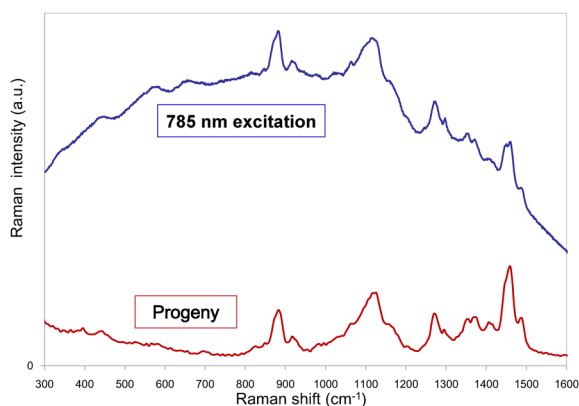
Fig. 7. Raman spectra of microcrystalline cellulose.

Fig. 8. Raman spectra of ethyl cellulose.

Fig. 9. Raman spectra of Shin-Etsu AQOAT.

widely used for tablets. However, inspections of cellulosic materials by traditional handheld Raman spectrometers with 532 nm or 785 nm excitation sometimes give us uncertain result because of featureless fluorescence background. Progeny with 1064 nm excitation is more suitable to identification of cellulosic materials. The following are comparisons of Progeny's spectrum excited at 1064 nm and a spectrum excited at 785 nm using a traditional handheld Raman spectrometer.

Figure 7 shows Raman spectra of microcrystalline cellulose[4] that is widely used in tableting as a filler, disintegrant and dry binder. The spectrum of 785 nm excitation has some deformations at the slopes of fluorescence background, but the spectrum of 1064 nm excitation does not have this problem.

Figure 8 shows Raman spectra of ethyl cellulose. The spectrum of 785 nm excitation has deformation by the influence of fluorescence in the high wavenumber region.

Figure 9 shows Raman spectra of Shin-Etsu AQOAT (hypromellose acetate succinate). The spectrum of 785 nm excitation has very high fluorescence, and Raman peaks are almost unobservable.
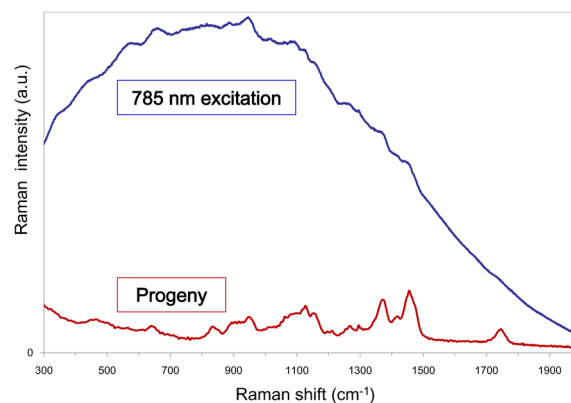
Table 1 shows library searching to identify six

different cellulosic excipients. Each excipient in a plastic bag was tested six times and compared to a user-made library. After each test, a sample bag is removed from the nose cone, then replaced, for making the reproducibility condition severe.

The large numerical characters are the numbers of times identified. The small numerical characters are cc values which show accuracy of identification. 1.0 means perfect match with the library item, and 0.97, 0.98 or 0.99 means almost perfect match with the library item. Though large particle sizes of some cellulosic excipients are detrimental to reproducibility, it can be seen from the table that all materials are identified correctly, with a high cc, every time. In other words, specificity of the analytical method in this material category is proven.

These correct identifications are possible only with 1064 nm excitation, as far as cellulosic excipients and other fluorescent materials are concerned.

3.2. Inorganic materials

Some of the pharmaceutical inorganic materials do not have a problem with fluorescence background, but other pharmaceutical inorganic materials have high fluorescence background if they are excited at 532 nm or 785 nm. Typical example of later case is talc, which is used for baby powder and others. Spectral comparison of talc between a traditional handheld Raman spectrometer with 785 nm excitation and Progeny with 1064 nm excitation is shown in Fig. 10.

3.3. Nutritional plants and herbal plants

Materials taken from plants are highly fluorescent. They are typical examples of materials which can be analyzed only with 1064 nm excitation. In this section, analyses of materials in herbal medicines with Progeny are described.

When analyzing materials in herbal medicines with Raman spectroscopy, we must pay attention not only to excitation wavelength but also to particle size, homogeneity and color of materials. Sometimes, particle size of herbal material is not small enough, and/or homogeneity is not good. Intentional defocusing of laser beam by utilizing the adjustable nose cone is

Table 1. Library searching of cellulosic excipients.

Real sample \ Library	HPMC	Shin-Etsu AQOAT	Ethyl cellulose	Hydroxypropyl cellulose	Low-substituted hydroxypropyl cellulose	Microcrystalline cellulose
HPMC	6 1.0, 1.0, 1.0, 0.99, 0.99, 0.99	0	0	0	0	0
Shin-Etsu AQOAT	0	6 0.99, 0.98, 0.98, 0.98, 0.97, 0.99	0	0	0	0
Ethyl cellulose	0	0	6 1.0, 1.0, 1.0, 1.0, 1.0, 1.0	0	0	0
Hydroxypropyl cellulose	0	0	0	6 0.99, 0.99, 0.99, 0.99, 0.99, 0.99	0	0
Low-substituted hydroxypropyl cellulose	0	0	0	0	6 0.99, 0.99, 0.99, 0.99, 1.0, 1.0	0
Microcrystalline cellulose	0	0	0	0	0	6 1.0, 1.0, 1.0, 1.0, 1.0, 1.0
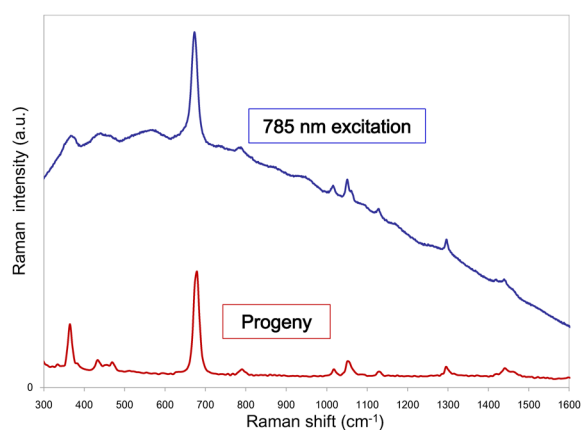
Fig. 10. Raman spectra of talc.
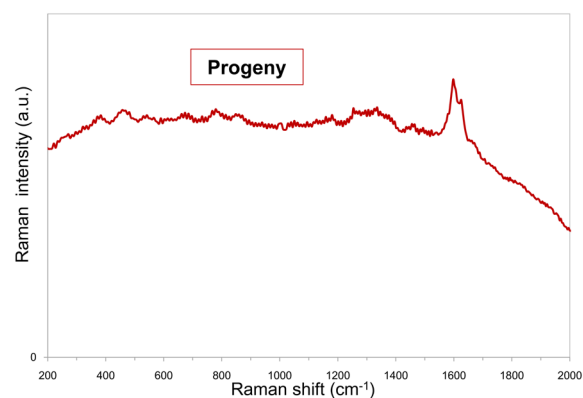
Fig. 12. Raman spectra of cinnamomi cortex.
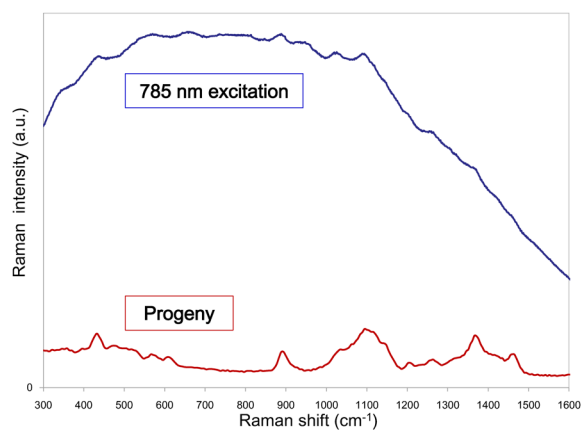
Fig. 11. Raman spectra of poria.
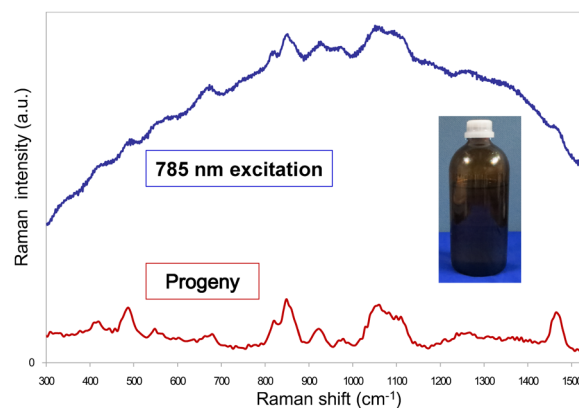
Fig. 13. Raman spectra of glycerol in amber color glass bottle.

often necessary. Another point is color of materials. When material color is dark brown, it absorbs laser light drastically, and rapid local temperature increase easily occurs. Further defocusing of the laser beam or reduction of laser power may be necessary.

Figure 11 shows Raman spectra of poria known as an herb for sedative effect and diuretic effect. The spectrum of 785 nm excitation has extremely high fluorescence, and Raman peaks are almost unobservable, whereas the 1064 nm excitation Raman spectrum collected with Progeny shows clear Raman peaks.

Figure 12 shows Raman spectra of cinnamomi cortex known as an herb for warming one's body. The Raman spectrum is collected with the Progeny and Raman peaks are seen. Fluorescence intensity was too high to prevent saturation of signal, when 785 nm excitation was used.

3.4 Test of liquid in amber color glass bottle

Figure 13 shows Raman spectra of glycerol in an amber-color glass bottle. Glycerol itself does not have a fluorescence problem. However, the amber-color glass bottle emits fluorescence depending on excitation wavelength.

The fluorescence from the glass bottle can be reduced by shifting the focal point to inside of the bottle, but the fluorescence of the bottle is still a problem if excitation wavelength is 532 nm or 785 nm. Progeny does not have this problem. Since many pharmaceutical liquid materials are kept in similar types of bottles to prevent the influence of room light, this is also an important advantage of Progeny's 1064 nm excitation.

4. Other important matters in RMID

In this report, advantages of 1064 nm excitation in RMID (raw material identification) were highlighted. However, when analytical instruments are used in the pharmaceutical industry or other GMP settings regulatory compliance is also very important in RMID. Progeny's software is truly compliant with 21 CFR part 11. An on-board digital camera and barcode reader provide error free data tracking. All data, spectral processing and library searches are stored together to meet required audit trails. Systems and procedures for IQ/OQ/PQ are well established. Also, customer support for method validation and other regulatory matters are now ready.

5. Conclusion

Fluorescence interference can be now minimized by the new technology incorporated in Progeny. By this progress, the applicability of handheld Raman spectrometers to different types of samples and sample conditions can be expanded. In the past, handheld Raman could not be used for some categories of samples. So, complementary analytical methods including laboratory level analytical methods were necessary. Now, Progeny can cover many types of samples by itself, and it increases total sample throughput. The authors believe the new technology and the new instrument will contribute to "inspection of samples in all containers" in the future.

References

(1) PIC/S GMP Guide, Annex 8.
(2) T. Nogami and F. Muta: *Rigaku Journal (English version)*, **29** (2013), No. 2, 16−18.
(3) H. Hisada, T. Nogami and F. Muta: *Journal of pharmaceutical machinery and Engineering*, **24**, (2015), No. 5, 407−413.
(4) M. Szymańska-Chargot, J. Cybulska and A. Zdunek: *Sensors*, **11** (2011), 5543−5560.