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Evaluation of the State of Active Ingredients in Pharmaceutical Preparations Using Fourier Transform-Raman Difference Spectra

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To examine the pharmaceutical application of Fourier transform (FT)-Raman spectroscopy, the state of active pharmaceutical ingredients (APIs) in a preparation of several forms was evaluated by investigating the Raman difference spectra between the preparation and excipient. The difference spectra indicated that APIs in alacepril tablets, caffeine sustained-release granules, and quinidine sulfate granules remained unchanged after the manufacturing process. However, the state of sparfloxacin in nanoparticles changed, although it remained unchanged in tablets or powders. These results show that the FT-Raman difference spectrum is expected to be utilized in the field of quality control of crystalline pharmaceutical preparations.

INTRODUCTION

An active pharmaceutical ingredient (API) in a preparation may change the crystalline condition or phase, or interact with excipients during the manufacturing process involving application of solvent heat, pressure, etc. Such a change in state may be critical if it affects the bioavailability of the active ingredient.[1,2] The crystalline condition of an API(s) in a preparation is generally examined by well known methods including powder X-ray diffractometry, infrared absorption spectrometry, and thermal analysis which are described in the *Japanese Pharmacopoeia XV* (JP 15). However, they are all destructive analyses, and the destruction process may sometimes result in a change in the state of an API in a preparation. Furthermore, in some cases, it is impossible to obtain useful information about the state of APIs with these methods due to the effects of excipients. With Raman spectroscopy, a preparation can be analyzed as it is, and thus the physicochemical conditions of APIs in the preparation remain unchanged. In addition, Fourier transform (FT)-Raman spectroscopy

is currently used for the quantitative determination of APIs[3—5] or the investigation of crystal polymorphism or amorphism of tablets.[6,7] In addition, Raman difference spectra are used to elucidate small structural differences in complex substances such as DNA and bisphenol-A-polycarbonate.[8—11]

Raman spectroscopy, a nondestructive analytical method, can be classified broadly into laser Raman spectroscopy, which uses ultraviolet or visible light, and FT-Raman spectroscopy, which uses near-infrared light.[12] Thus, when the former is applied to analysis of pharmaceutical preparations, a sample in the form of, for example, a tablet may be heated and sometimes burned by excitation light with strong energy. The latter type of FT-Raman spectroscopy cause fewer such problems than laser Raman spectroscopy and is considered to be more suitable for the analysis of pharmaceutical preparations. Therefore, in this study, we examined the state of APIs in pharmaceutical preparations using FT-Raman spectroscopy, specifically the FT-Raman difference spectrum between the spectrum of preparation and that of excipient.

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EXPERIMENTAL

Sample Materials   Alacepril tablets, sparfloxacin tablets, powders, and nanoparticles, quinidine sulfate particles, and caffeine sustained-release particles were provided by Dainippon Sumitomo Pharma Co., Ltd. The ingredients for core granules or coating (excipients), such as poly-L-lactic acid (average molecular weight 2290), were also provided by Dainippon Sumitomo Pharma Co., Ltd.

Apparatus and Measurement   FT-Raman spectra were measured on a JASCO FT/IR 800 near FT-infrared spectrophotometer (Jasco Co., Ltd., Tokyo, Japan) with an FT-Raman attachment (Jasco RFT-800). Measurements were carried out using as the elicitation line of a PSU-S-1064 YAG laser (1064 nm, IE Optomech Ltd., UK) with laser power of about 1.0 W and resolution of $4\ cm^{-1}$ (basically 100 scans). FT-Raman spectroscopy was conducted by placing a sample tablet or powder on a slide glass, while adjusting the position with the built-in video camera of the RFT-800.

For the measurement of difference spectra, FT-Raman spectra of the pharmaceutical preparation, API, and a mixture of excipients were compared, and an independent band(s) specific to the excipient and not present in the API spectrum was selected. Then the difference spectrum was obtained by processing the data using four basic calculations of the arithmetic program in such a manner that the independent band of the excipient was eliminated from the spectrum of the sample. The resulting difference spectrum and the original spectrum of the API were compared.

The FT-infrared spectra were measured on an Impact 400 infrared spectrophotometer (Nicoret Co., Madison, WI, USA) using the KBr disk or Nujol method as described in JP 15. KBr disks were prepared using a Jasco tabletting machine (10 mm diameter) and R-16B-02-type pressurizer (Riken Seiki Co., Ltd., Niigata, Japan).

Thermal analysis was performed on a Shimadzu DSC-50 Differential Scanning Colorimeter (Shimadzu Co., Ltd., Kyoto, Japan).

Reagents   Reagents such as hydrochloric acid and lactic acid were of reagent grade (Nacalai Tesque, Kyoto, Japan). KBr and liquid paraffin (Nujol) for IR were purchased from Jasco Co., Ltd. and Merk AG (Germany) and used without further processing.

RESULTS AND DISCUSSION

Alacepril Tablets, Caffeine Sustained-release Particles, and Quinidine Sulfate Particles   Figures 1, 2, and 3 show the difference spectrum, Raman spectrum of caffeine hydrate, and Raman spectrum of alacepril tablets (40% API in four excipients), caffeine sustained-release particles (17% active ingredient in five excipients), and quinidine sulfate particles (50% API in three excipients), respectively. In every case, the difference spectrum is almost identical to the spectrum of the API. These results indicate that the state of the API such as crystalline condition was not changed during the manufacturing process in these preparations and that the difference spectrum is use-
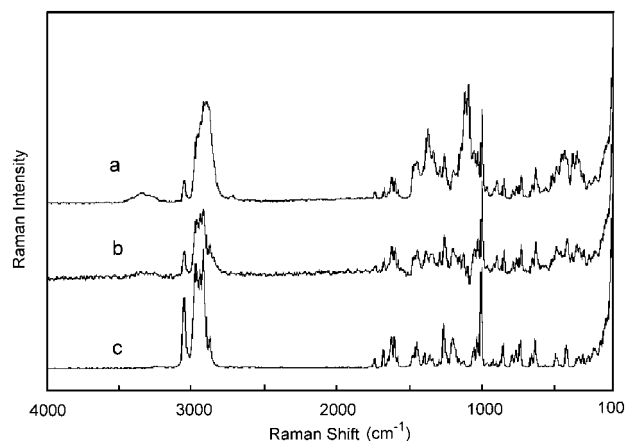

Fig. 1.   FT-Raman Spectrum of Alacepril Tablet (a), Difference Spectrum from Spectrum (a) to Spectrum of a Mixture of Four Excipients (b), and Spectrum of Alacepril (c)
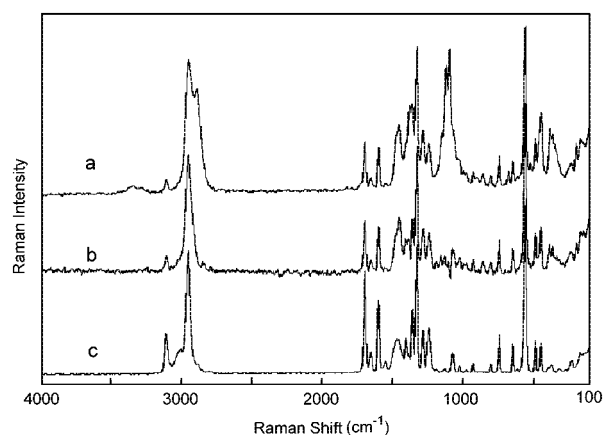

Fig. 2.   FT-Raman Spectrum of Caffeine Sustained-release Particles (a), Difference Spectrum from Spectrum (a) to Spectrum of a Mixture of Three Excipients (b), and Spectrum of Caffeine Hydrate (c)
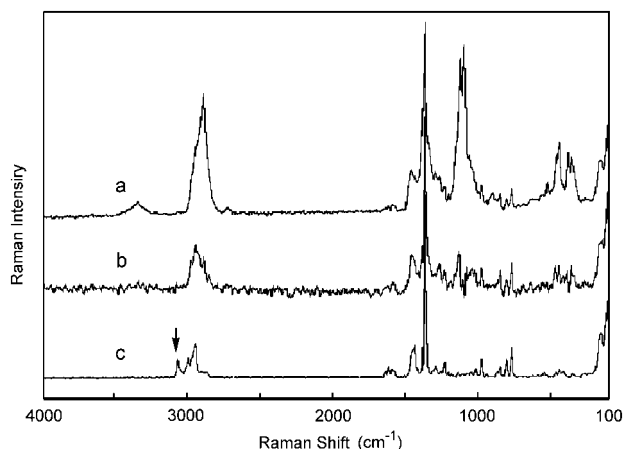
Fig. 3. FT-Raman Spectrum of Quinidine Sulfate Particles (a), Difference Spectrum from Spectrum (a) to Spectrum of a Mixture of Five Excipients (b), and Spectrum of Quinidine Sulfate (c)

↓ indicates a Raman band observed only for quinidine sulfate.

Fig. 4. Structure of Sparfloxacin

ful to obtain the FT-Raman spectrum of an API in a preparation. In the spectrum of quinidine sulfate, there is a peak at a different position from that in the difference spectrum (indicated by an arrow); however, it should be unrelated to a change in state because the wave numbers of remaining peaks are almost the same. In particular, the wave numbers of the quinidine sulfate peaks in the fingerprint region agree with those obtained from the difference spectrum. Thus we concluded that quinidine sulfate also did not undergo a change in state during the manufacturing process.

Sparfloxacin Tablets, Powders, and Nanoparticles Sparfloxacin is a compound with the formula shown in Fig. 4 and has potent antibacterial activity. In this study, three formulations, *i.e.*, tablets, powders, and nanoparticles, were examined because preparations containing sparfloxacin as an API are generally in those forms. The tablets contain 63% sparfloxacin and 12 excipients, the powders 20% sparfloxacin and seven excipients, and the nanoparticles 8% sparfloxacin and one excipient, *i.e.*, poly-L-lactic acid. Despite the relatively low concentration of the API, nanoparticles were expected to be suitable for the measurement of difference spectra because they contain only one excipient. A good difference spectrum of the nanoparticle preparation was obtained as expected.

The difference spectra of the three preparations and Raman spectrum of sparfloxacin are shown in Fig. 5. The difference spectrum of nanoparticles differs from the spectrum of sparfloxacin. An especially marked
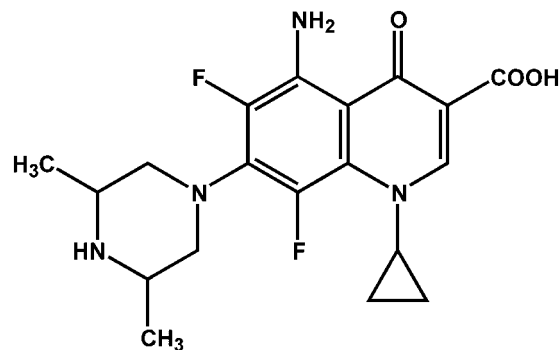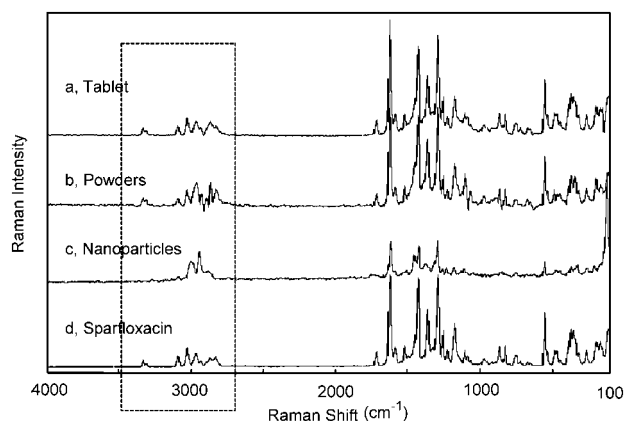

Fig. 5. FT-Raman Difference Spectra from Sparfloxacin Tablet (a, Tablet), Powders* (b, Powders), Nanoparticles (c, Nanoparticles), and FT-Raman Spectrum of Dry Sparfloxacin (d, Sparfloxacin)

*20% sparfloxacin in a mixture of lactose, hydroxypropyl cellulose, talc, low-polymerized hydroxypropylcellose LH31, hydroxypropylcellose, acetylmonoglyceride and Aquacoat (a 88.1: 4.2: 7.7 mixture of ethylcellose, sodium lauryl sulfate, and acetylalcohol).

difference was found within the region indicated by a dashed line in Fig. 5. In addition, there were differences among spectra regarding wave number, even in the region subsequent to 1700 cm^{-1}. Accordingly, in nanoparticles, sparfloxacin possibly underwent some changes due to procedures during manufacturing or measurement. We then investigated the causes of the changes.

First, we examined whether the elevated temperature due to heating during the manufacturing process of preparations or laser irradiation exercised any influence on the state of sparfloxacin. When sparfloxacin was heated to 200℃ and cooled and the FT-Raman and FT-IR spectra were measured, no changes were observed in the spectra. Sparfloxacin was then examined using differential scanning calorimetry (DSC), which only gave a peak at 269℃, correspond-

ing to the melting point. It was therefore considered that crystal polymorphism was not present. These results indicate that the change in spectrum observed in the difference spectrum of nanoparticles did not result from the alteration of the crystal form by heat. Further, a tablet was prepared by compressing sparfloxacin bulk powder under pressure of 1000 N/cm^2, pulverizing the resultant tablet, and subjecting it to IR and FT-Raman spectroscopies. There were no changes due to pressurization in the spectrum.

Then the influence of moisture absorption during the manufacturing process or preservation was examined. Dry sparfloxacin was kept in a desiccator saturated with water at room temperature for 1 week or 1 month and then the FT-Raman spectrum was measured. The resulting Raman spectrum was different from that of dry sparfloxacin due to the formation of trihydrate, but it did not agree with the difference spectrum either. It was therefore concluded that the change in spectrum observed in the difference spectrum of nanoparticles did not result from moisture absorption.

The excipients used in these three forms of preparations were then compared. Tablets contained corn starch, hydroxypropyl cellulose, etc., and powders lactose, hydroxypropyl cellulose, talc, etc. However, nanoparticles contained only poly-L-lactic acid as an excipient, which showed the spectrum change for sparfloxacin as described above. This led to the presumption that the interaction between sparfloxacin and poly-L-lactic acid may be involved in the change in spectrum. Sparfloxacin and poly-L-lactic acid were then pulverized in an agate mortar at different mixing ratios and subjected to Raman spectroscopy. Some of the resultant spectra are shown in Fig. 6. As seen in Fig. 6, the FT-Raman spectrum gradually changes as the ratio of sparfloxacin to poly-L-lactic acid is lowered and becomes almost the same as the difference spectrum of the preparation (nanoparticles) when the ratio decreases to 8%. This indicates that sparfloxacin possibly interacts with poly-L-lactic acid in a concentration-dependent manner. Sparfloxacin is a basic substance and poly-L-lactic acid contains a lower molecular-weight substance. Therefore sparfloxacin could interact with the terminal carboxylic acid of poly-L-lactic acid. To confirm this assumption, interaction between sparfloxacin and acids was investigated.

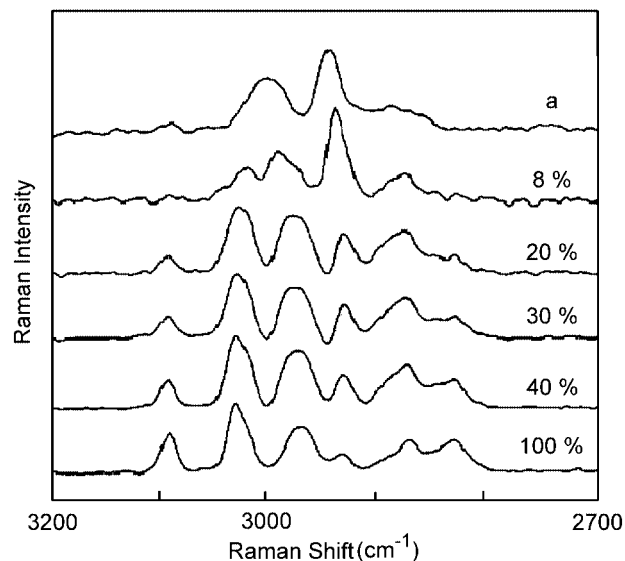Sparfloxacin was dissolved and mixed with 1 mol/l

Fig. 6. FT-Raman Difference Spectra from Sparfloxacin Nanoparticles (a), and 8, 20, 30, and 100% Sparfloxacin in Poly-L-lactic Acid

of hydrochloric acid, lactic acid, or a mixture thereof, evaporated to dryness under reduced pressure over sodium hydroxide, and subjected to measurement of FT-Raman and FT-IR spectra. The resulting FT-Raman spectra were in agreement with each other except for the region of 3200—2700 cm^{-1}, where the acid added affected the spectrum. When sparfloxacin was treated with the mixture of hydrochloric and lactic acids, the FT-Raman spectrum in this region was consistent with the difference spectrum shown in Fig. 5 (a).

Figure 7 shows the FT-Raman spectrum of sparfloxacin within the region of 1800—1000 cm^{-1} obtained by dissolving sparfloxacin in hydrochloric acid, evaporating the solution *in vacuo* to dryness, and measuring the FT-Raman spectrum. Figure 7 indicates that the FT-Raman spectrum of sparfloxacin after treatment with 1 mol/l of hydrochloric acid was almost in agreement with the difference spectrum of nanoparticles. It was determined that sparfloxacin underwent protonation when manufacturing nanoparticles.

In conclusion, the FT-Raman spectrum, a nondestructive analysis, is useful to obtain information regarding the state of APIs in preparations based on difference spectra. The difference spectrum of FT-Raman spectroscopy is expected to be utilized widely in the field of quality control for crystalline conditions of pharmaceutical preparations.
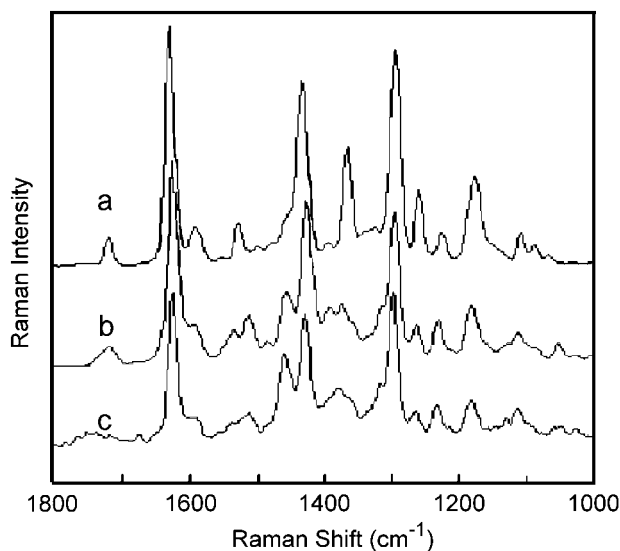
Fig. 7. FT-Raman Spectra of Dry Sparfloxacin (a), and Sparfloxacin after Treatment with 1 mol/l of HCl (b), and Difference Spectrum from Sparfloxacin Nanoparticles (c)

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