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Monitoring of crystalline transition during wet granulation process using probe type low frequency Raman spectrometer

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ABSTRACT

In recent years, real time analysis has been widely studied using various Process Analytical Technology tools particularly in the quality control of drug products. In this study, we investigated the improvement of sensitivity and the reduction of measurement time using a low frequency (LF) Raman spectrometer equipped with a new light source for *in situ* monitoring of crystalline transformation of active pharmaceutical ingredients (API) during wet granulation process. Three model drugs were chosen to assess the system sensitivity, hydrate transition, and cocrystal dissociation. LF Raman spectra showed the specific peaks for crystalline indomethacin and theophylline which were typical and well known for Raman active APIs. The laser power was strengthened from 58 to 300 mW, thus the measurement time can be reduced nearly 500 times which was significantly faster than the old probe. In addition, signal to noise ratio was drastically improved as seen from the clear signal intensity of API with low interference from the excipients. Obvious peak shifts were observed in the spectra of LF region reflecting the dissociation of CAF cocrystal. These three model drugs suggested that LF Raman spectroscopy is preferable for specific detection of crystalline APIs.

Keywords: Low-frequency, Raman spectroscopy, wet granulation, crystalline transition

INTRODUCTION

In quality control of APIs, optimization of manufacturing processes based on scientific evidence has been emphasized.[1-3] Many research studies of quality controls using PAT (Process Analytical Technology) tools have been conducted, without sampling, thus it is possible to measure all products in each manufacturing process and analyze their quality in real time. Wet granulation is expected to ensure uniformity of ingredients and to sustain formulation efficacy by adding a binder such as water to the drug substance. However, the added water may change the crystal form, for example, to drug in the hydrate form, which then causes a major effect on the manufacture, quality, and efficacy of the drug product. Low frequency (LF) Raman spectroscopy has several advantages over other PAT tools, such as non-contact and non-destructive measurement with less influence of moisture and no pre-treatment required.[4] LF Raman spectra provide information on the crystal lattice structure which is useful for identifying crystal polymorphs while CV Raman spectra provide chemical structure information.[5] In the previous studies, we used LF Raman spectroscopy to investigate the crystal structure and monitored the crystal form during wet granulation.[6] However, some limitations of the monitoring and measurement were found, namely, the noise in the spectrum interfering the detection of API peaks and the long exposure time to the laser measurement to eliminate peak noise, which then may modify or destroy samples. In this study, we verified crystalline states during wet granulation using LF Raman spectroscopy equipped with a new light source and compared with the

conventional (CV) Raman spectrometer. Three model drugs (indomethacin, theophylline, and caffeine cocrystal) were used to ensure the improvement of detection sensitivity and measurement efficiency.

MATERIALS AND METHODS

Materials

Indomethacin (IND) and theophylline form II (TP form II) (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan; TCI); theophylline monohydrate (TP MH) (Thermo Fisher Scientific, Massachusetts, U.S.); and caffeine form II (CAF form II) and caffeine monohydrate (CAFMH) (FUJIFILM Wako Pure Chemical Industries Co., Ltd., Osaka, Japan; Wako) were purchased. Glutaric acid (GA) (TCI) was used as a coformer. The followings were used as excipients in wet granulation: Microcrystalline cellulose (MCC) (Asahi Kasei Corp., Tokyo, Japan; Asahi Kasei) and hydroxypropyl cellulose (HPC) (Wako). CAF/GA cocrystal was prepared as the publication[7] and confirmed the cocrystal formation by powder X-ray diffraction measurement [Table 1].[7]

Raman Spectroscopy

LF Raman spectra were obtained during wet granulation using a THz-Raman® probe system comprising a TR-PROBE (Coherent Inc., Monrovia, CA, USA) attached to an All In One Raman spectrometer (MarqMetriX LLC, Seattle, WA, USA). CV Raman spectra were obtained using a BallProbe attached to an All In One Raman spectrometer. The sample spot size was approximately 100 μm. The measurement conditions of the All In One Raman system were that exposure time was 0.3 s and the laser power was 300 mW. On the other hand, the previous Raman system was 148 s and 58 mW.[6] After calibrating the Raman shifts using sulfur, the spectra were acquired with 4 cm^{-1} resolution using HoloGrams software version 4.1 (Kaiser Optical Systems Inc.).

Settings of the Granulation Process

Granulation was performed under the same conditions, referring to the publication.[6] The rotation speed of the impeller and chopper was set at 200–300 rpm and 1000–2000 rpm. A Raman probe was attached to the granulator. Three model drugs (IND, TP, and CAF/GA cocrystal) were selected for the purposes of three different assessments during wet granulation in the presence of excipients. MCC (62 or 77% w/w of formulation) and HPC (fixed at 3% w/w of formulation) were used as the main granulation excipients. To investigate whether the crystalline form of the APIs can be measured without being affected by moisture and excipients, IND, which is known as a poorly water-soluble drug, was used (20% IND with 3% HPC and 77% MCC.) The total addition of purified water was 30 mL in a granulator. To examine the transition to hydrate, TP form II, HPC, and MCC were mixed in proportions of 20%, 3%, and 77%, respectively. At the end, 50 mL of water were added to the powder mixture. To monitor the cocrystal dissociation, CAF/GA cocrystal (35%), HPC (3%), and MCC (62%) were mixed with the addition of 40 mL water in a granulator.

Table 1: Formulation design

Formulation (%w/w) - Monitoring of	IND	TP form II	CAF-GA*	MCC	HPC
API	20	-	-	77	3
Hydrate transition	-	20	-	77	3
Cocrystal dissociation	-	-	35	65	3

*Equivalent to CAF 20%w/w

RESULTS AND DISCUSSION

Comparison of the Measurements Using Two Different LF Raman Spectrometers

The LF Raman spectra of MCC and HPC used as wet granulation excipients are considerably weaker than those of crystalline APIs [Figure 1a], so LF Raman probe can be used for *in situ* monitoring of drugs without excipient peak interference. No change in IND crystal form was observed over 40 min granulation with the constant and characteristic peaks intensity (30, 48, 70, and 98 cm^{-1}) [Figure 1b]. This indicated that LF Raman detection of API was not affected by water addition. Compared to the previous LF Raman device, the laser power was strengthened from 58 to 300 mW in the present study and the measurement time reduced nearly 500 times (from minutes to milliseconds) which means that more data and more accurate monitoring could be obtained over the process. In addition, signal to noise ratio was drastically improved as seen from the clear signal intensity of crystalline API with low interference from the excipients [Figure 1b and c].

As of monitoring TP form II, the specific peaks of TP form II (32, 66 cm^{-1}) disappeared with the addition of water, while the new peaks specific to TP MH (64, 92 cm^{-1}) was observed, suggesting the presence of hydrate transition during processing [Figure 2a,b]. Furthermore, wet granulation using CAF/GA cocrystal can be monitored at the specific peak of 32 cm^{-1}; however, the 32 cm^{-1} peak changed with the addition of water, and was finally replaced with the new peaks of CAF form II (20 cm^{-1}) and CAF MH (66, 80 cm^{-1}), which imply the dissociation of cocrystal [Figure 2d,e]. The incident was confirmed by PXRD as shown in Figure 3. The peaks of CAF form II (12 and 27°) and CAF MH (10, 12, and 27°) mixture were found after wet granulation. Like the previous case, clearer peaks of the polymorph and cocrystal conversion were obtained [Figure 2b and e] than those from the previous LF Raman system [Figure 2c and f]. Wet granulation of the three crystal forms can be proof-of-concept for real time monitoring using a high power LF Raman probe and emphasized the improvement of measurement sensitivity, potentially leading to the higher standardized quality control of APIs.

Comparison of the Measurements Between LF Region and CV Region Raman Spectroscopy

The results of IND crystal form monitoring in LF and CV region indicated that the specific peaks of IND were measured all times without being affected by moisture. On the other hand,

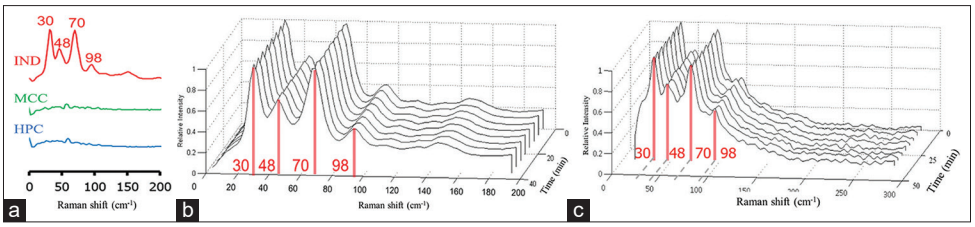

Figure 1: LF Raman spectra of (a) IND, MCC, and HPC under dry condition and during wet granulation of IND obtained from (b) present system, and (c) previous system

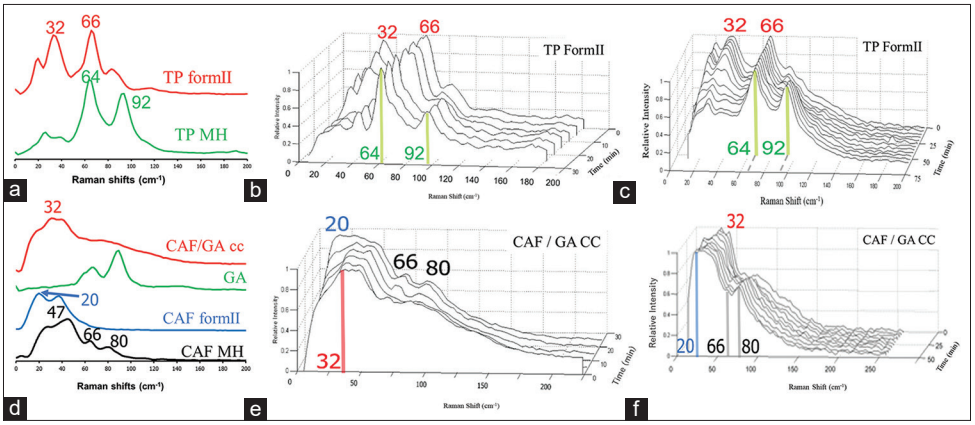

Figure 2: LF Raman spectra of (a) TP form II and TP MH, (d) CAF/GA cocrystal, GA, CAF form II, and CAF MH under dry condition. LF Raman spectra during wet granulation of TP form II and CAF/GA cocrystal obtained from (b) (e) present system and (c) (f) previous system

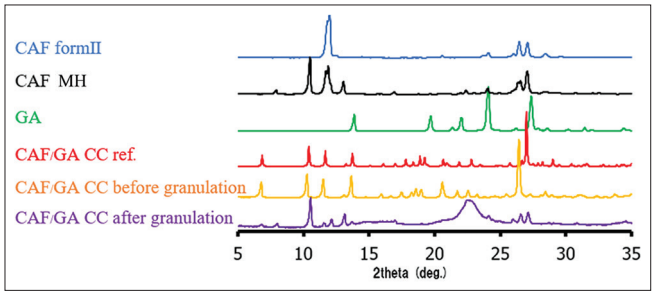

Figure 3: PXRD patterns of CAF form II, CAF MH, GA, CAF/GA cocrystal ref, and CAF/GA cocrystal before granulation and CAF/GA cocrystal after granulation

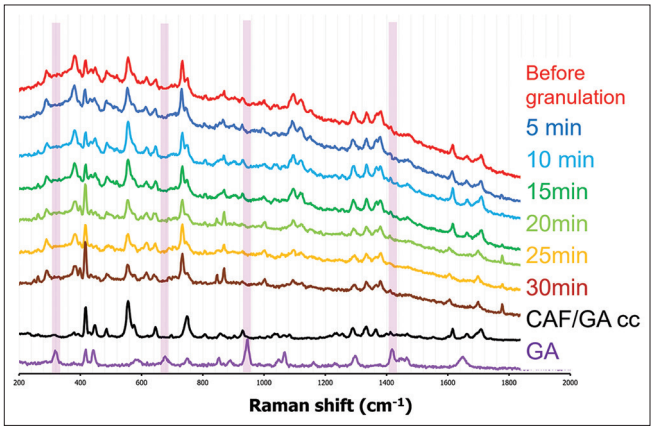

Figure 4: CV Raman spectra during granulation of CAF/GA cocrystal

wet granulation of TP form II was affected by added water and detected the transformation to TP MH. These two cases demonstrated the possibility of using CV Raman spectroscopy to monitor the hydrate transition (data not shown). On the contrary, Figure 4 showed that there was no difference among all the peaks of CAF/GA cocrystal in CV region from the start to the end of granulation whereas dissociation of the cocrystal after wet granulation was detected by PXRD (data not shown). The CV Raman peaks of CAF/GA cocrystal, CAF form II, and CAF MH were almost matched [Figure 5].

Monitoring wet granulation of CAF/GA cocrystal and cocrystal dissociation can be misinterpreted from the peak similarity of all the CAF crystal forms; therefore, the characteristic peaks of GA were selected to monitor the dissociation instead (as purple-highlighted shown in Figures 4 and 5). However, two main limitations that the measurement could not monitor the cocrystal by CV Raman are likely due to the peak of the excipients[8] and the weak intensity of GA peak. Unlike the LF region, the peaks of excipients were measured in CV region [Figure 5] because CV Raman scattering is derived from a functional group of all chemical compounds.[5] Therefore, the excipient peaks overlap with the specific peaks of GA, which then affect the detection of the GA peak. Another reason is the peak intensity of GA was approximately 5 times weaker than CAF MH, hence the characteristics peaks of GA were masked and cannot be used as a peak indicator of the cocrystal dissociation.

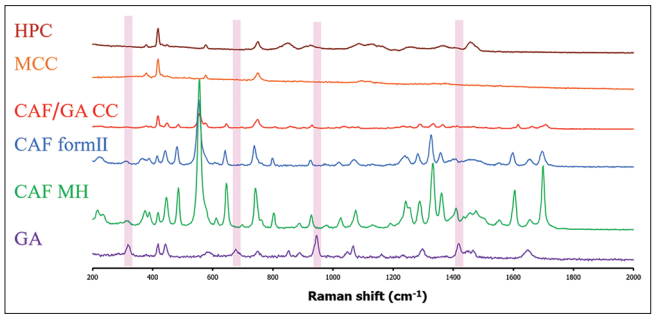

Figure 5: CV Raman spectra of HPC, MCC, CAF/GA cocrystal, CAF form II, CAF MH, and GA

CONCLUSION

The LF and CV Raman probe can monitor the change in crystal from without the interference of water addition during wet granulation. By performing measurement using a spectroscope with a strong laser power, it was possible to improve the detection sensitivity and shorten the measurement time. The peaks of APIs were mainly detected in the LF region, whereas those of both API and excipients were detected in the CV region which can affect the identification of APIs. In this study, it was suggested that LF Raman spectroscopy is preferable for specific detection of crystalline APIs.

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