

Laboratory real-time and *in situ* monitoring of mechanochemical milling reactions using Raman spectroscopy**

Davor Gracin, Vjekoslav Štrukil, Tomislav Friščić, Ivan Halasz, * Krunoslav Užarević*

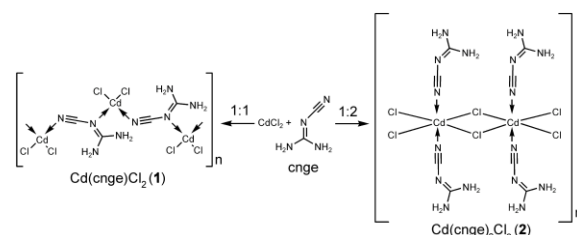
Abstract: Mechanistic understanding of mechanochemical reactions is sparse and has been acquired mostly by stepwise *ex situ* analysis. We describe here an unprecedented laboratory technique to monitor the course of mechanochemical transformations at the molecular level *in situ* and in real time by using Raman spectroscopy. The technique, which employs translucent milling vessels that allow collecting a Raman scattering signal from the sample as it is being milled, was validated on mechanochemical reactions forming coordination polymers and organic cocrystals. The technique enabled assessing reaction dynamics and course under different reaction conditions as well as, for the first time, direct insight in the behaviour of liquid additives during liquid-assisted grinding.

Mechanochemical reactions^[1] are traditionally devoted to processing of inorganic materials,^[2] but have recently been recognised as a powerful route to conduct transformations of organic,^[3] pharmaceutical^[4] and metal-organic materials^[5] in a selective, energy- and atom-efficient, as well as clean manner.^[6] However, understanding of mechanisms of mechanochemical reactions remains poor, primarily because such reactions are conducted in sealed, rapidly moving reaction chambers (milling jars) and involve rapid and violent motion of the milling media (milling balls), severely hindering attempts to directly monitor the reaction course. For instance, while the accelerating and templating effect of liquid and ionic additives is well documented,^[1a,1c,1d,6a] little is known about the underlying mechanisms of such liquid-assisted grinding (LAG)^[7] and ion- and liquid-assisted grinding (ILAG) processes.^[8] Usually, mechanistic studies of mechanochemical reactions are performed in a stepwise manner,^[9] where milling is interrupted at designated time intervals and the milling vessel opened in order to sample the reaction mixture for *ex situ* analysis. This unavoidably disturbs the reaction conditions and involves an inherent delay between sampling and analysis. Thus, such analysis is not suited for a number of mechanochemical processes, such as self-sustained reactions,^[10] reactions where the reaction mixture contains volatile components, as well as rapidly transforming crystalline or amorphous^[11] intermediates. Recently, a method was presented for

real-time and *in situ* monitoring of milling reactions by diffraction of high-energy synchrotron X-rays.^[12] This method proved powerful in detecting bulk crystalline materials, their transformations and evolution of the reaction mixture composition without the need to interrupt the milling process.^[13,14]

However, besides requiring access to a synchrotron radiation source, X-ray diffraction is not a straightforward method for characterising reaction mechanisms at a molecular level, or for characterising amorphous phases which may occur during milling.^[11,14] Here we describe a laboratory real-time and *in situ* monitoring technique that employs translucent milling jars made from poly(methyl)methacrylate (PMMA, also known as Perspex or Plexiglas)^[12] to obtain a time-resolved Raman scattering signal from a milled sample without the need to interrupt the milling process. In contrast to X-ray diffraction which is largely limited to crystalline materials, Raman spectroscopy will provide information on immediate molecular surroundings within a sample regardless if it is in a rigid crystalline, amorphous or liquid phase. Indeed, Raman spectroscopy has often been utilised to obtain *in situ* spectroscopic information^[15] in a wide range of systems and became particularly attractive with the introduction of portable Raman spectrometers.^[16,9c] Recently, James and co-workers have demonstrated the use of Raman spectroscopy to quantitatively monitor the mechanochemical formation of a metal-organic framework.^[9b] However, since conventional steel milling assembly was used therein, the authors had to resort to stepwise *ex situ* analysis and a low-vapour-pressure liquid as is N,N-dimethylformamide.

Scheme 1. Mechanochemical reactions between CdCl₂ and cnge.



As the first model system, we have investigated the previously reported neat grinding (NG) and LAG reactions of cadmium chloride (CdCl₂) and cyanoguanidine (cnge).^[17] Depending on the ratio of CdCl₂ and cnge, two coordination polymers are known to form: the three-dimensional (3-D) material Cd(cnge)Cl₂ (1) and the one-dimensional (1-D) Cd(cnge)₂Cl₂ (2) (Scheme 1). *Ex situ* powder X-ray diffraction (PXRD) showed that the 3-D polymer 1 is formed by brief neat grinding (up to 10 mins) using either two small balls (1.3 g each) or one heavier ball (4.0 g), regardless whether the initial ratio of CdCl₂ and cnge reactants was 1:1 or 1:2. The 1-D polymer 2 was more difficult to obtain and required longer milling with a 4-gram ball.

The changes in Raman spectra as the reaction takes place are expected to be most pronounced in the region below 300 cm⁻¹, where the stretching vibrations involving cadmium are positioned, and in the region from 2100 cm⁻¹ to 2250 cm⁻¹ where the nitrile group vibrations lie (Figure 1a).^[17b] *In situ* monitoring of the neat grinding of CdCl₂ with either 1 or 2 equivalents of cnge using two small balls revealed surprisingly, only a mixture of reactants even after 40 min milling (Figs. 1b and 1c). However, *ex situ* analysis of the reaction mixtures

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3 days later (by Raman spectroscopy and PXRD) revealed pure **1** resulting from the 1:1 starting mixture and a mixture of **1** and **cnge** resulting from the 1:2 starting mixture. Thus, it is likely that **1** was previously formed not by milling of the reaction mixture, but upon its subsequent aging. To test whether the observed difference in behaviour from the previous study may have been caused by the difference in milling jar material, we repeated the reactions in a steel milling assembly. After 25 minutes milling the steel vessels were opened and the reaction mixtures rapidly (within seconds) analysed by Raman spectroscopy, which revealed only a mixture of reactants. However, subsequent aging of these mixtures resulted in formation of **1** (Figs. S9 and S10). We found that the aging time required for formation of **1** greatly varied from sample to sample, ranging from minutes to days, and is probably influenced by humidity.

Switching to a heavier ball (4.0 g) resulted in the formation of **1** from a 1:1 mixture of reactants (Fig. 1d). Approximately 10 minutes into the milling, the band at 228 cm^{-1} increases in intensity and gradually shifts towards 240 cm^{-1} finally shaping the three characteristic bands of **1**. This is accompanied by a gradual decrease of the **cnge** band at 2158 cm^{-1} and emergence of bands at 2169 cm^{-1} and 2208 cm^{-1} as **cnge** is coordinated to cadmium forming **1**. The reaction is complete after ca. 30 minutes.

From the 1:2 mixture of CdCl_2 and **cnge**, polymer **2** was previously obtained by neat grinding only when a 4 g ball was used. Furthermore, attempts to investigate the formation of **2** by *ex situ* analysis were unsuccessful, as dividing the milling process into 5-minute periods led to the persistent formation of **1**. This led to a proposal that **1** is an intermediate in the synthesis of **2**. In contrast, *in situ* Raman spectroscopy monitoring reveals that **2** can form directly from the reactants after 35 minutes milling, and its formation is complete after 60 minutes (Fig. 1e). Formation of **2** was followed by the emergence of the band at 253 cm^{-1} . The nitrile group vibrations in **2** are similar as in pure **cnge** but shifted by ca. 6 cm^{-1} to higher wave numbers. Remarkably, when a ball of 2.9 g weight was used the time-resolved spectra revealed the formation of **1** after 27 minutes, which then reacted with the remaining **cnge** to yield pure **2** after ca. 40 minutes milling (Figs. S14-S17).

The above described comparison of *in situ* studies with results of earlier *ex situ* investigations clearly shows that grinding reactions are highly sensitive to reaction conditions and sample manipulation. As a result, *ex situ* analysis may lead to misleading results, highlighting the benefit of the herein presented laboratory methodology to assess the reaction course without interrupting the milling process.

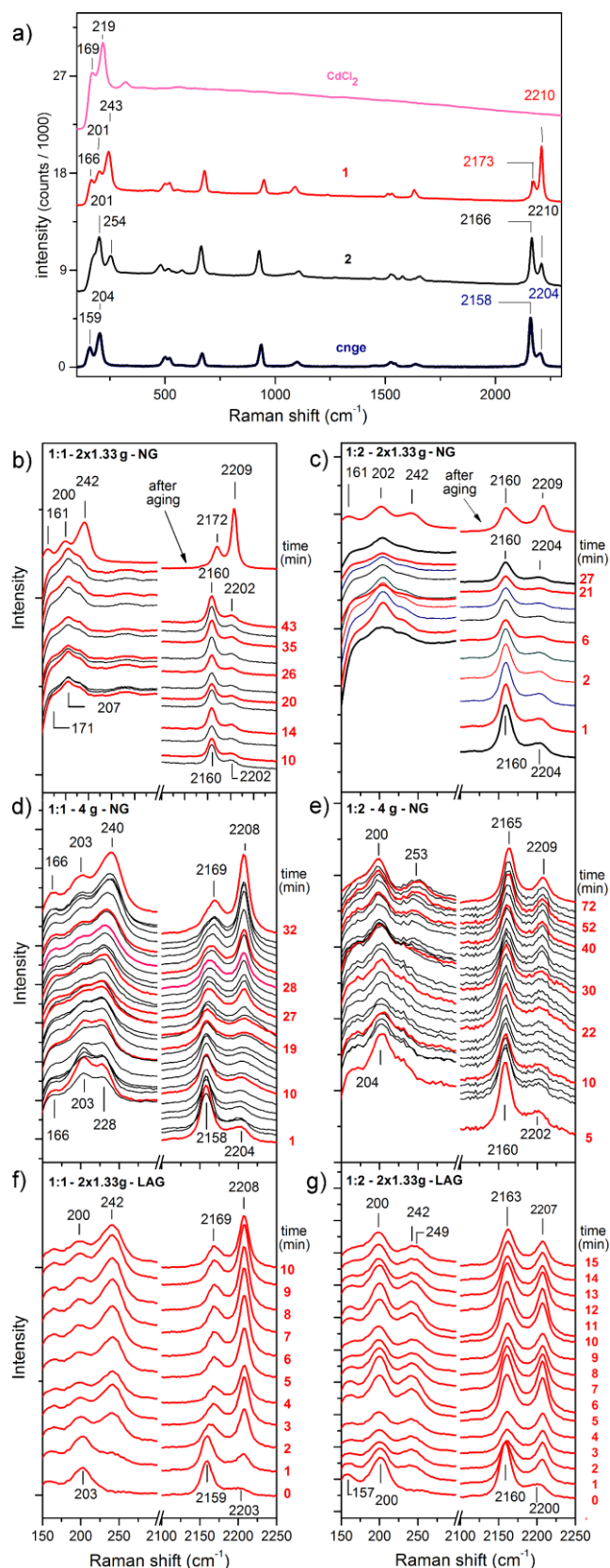


Figure 1. a) Raman spectra of pure reactants and two products. Time resolved Raman spectra of NG of 1:1 and 1:2 reactant mixture using two small balls (b and c, respectively), NG of 1:1 and 1:2 reactant mixture using one heavier ball (d and e, respectively) and LAG of 1:1 and 1:2 reactant mixture using two small balls (f and g, respectively). Wavenumbers of characteristic bands are given in the spectra.

Next, LAG reactions of CdCl_2 and cngc were performed using two small balls (1.3 g each) and methanol (MeOH) as the grinding liquid. Raman spectroscopy allowed, for the first time, direct investigation of the behaviour of the added liquid during milling. Vibration bands of MeOH (C–O stretch at 1034 cm^{-1} and C–H stretch at 2841 cm^{-1}) did not change significantly as compared to pure methanol. The MeOH bands are clearly visible in the spectra when the mixture becomes homogenous (ca. 30 seconds into milling) and do not change their position and intensity during milling (Figs. S18 and S20), indicating that MeOH does not actively participate in the mechanochemical reaction. However, MeOH significantly enhanced reactivity (Figs. 1f and 1g), allowing the synthesis of pure **1** from a 1:1 mixture of CdCl_2 and cngc after only 4 minutes. Such a rapid transformation could not readily be monitored using the earlier *ex situ* PXRD technique.^[17] Similarly, LAG of 1:2 mixture of CdCl_2 and cngc almost immediately yielded **1** as an intermediate. Formation of **1** in a mixture with residual cngc is recognised by the two bands belonging to the nitrile group becoming similar in intensity, as well as by the emergence of a band at 240 cm^{-1} . Subsequent milling yielded **2**, as evidenced by the emergence of the bands at 250 cm^{-1} and 1578 cm^{-1} , and by the change in relative intensities of the two nitrile vibration bands.

Next, we investigated the use of *in situ* Raman spectroscopy reaction monitoring to follow the course of a model pharmaceutical cocrystals of nicotinamide (na) and suberic acid (sub). Depending on the reactant ratio, two cocrystals may be formed: the 1:1 cocrystal (na)(sub) (**3**) and the 2:1 cocrystal (na)₂(sub) (**4**) (Fig. 2a).^[14,18] Previous *in situ* synchrotron X-ray diffraction monitoring revealed the direct formation of **3** from the 1:1 mixture of na and sub, as well as the stepwise formation of **4**, via the intermediate **3**, from a 2:1 mixture of na and sub.^[14] The outcomes of *in situ* Raman spectroscopy monitoring were challenging to analyse since the reactants and the two cocrystals have very similar Raman spectra (Fig. 2b). Nevertheless, we were able to monitor the consumption of reactants and clearly establish the formation of pure **3** from the 1:1 na:sub mixture in the spectrum obtained after ca. 40 minutes of neat milling. The consumption of na could also be followed through gradual disappearance of the two bands belonging to C–H stretching vibrations at 3069 cm^{-1} and 3102 cm^{-1} , as well as of the carbonyl stretching band at 1676 cm^{-1} (Fig. 2c).

The analysis is further complicated when both **3** and **4** may occur simultaneously. Supramolecular architectures in **3** and **4** are comprised of similar supramolecular synthons (Fig. 2a), resulting in almost identical Raman spectra. Most pronounced differences in the spectra of the two cocrystals can be found in bands belonging to ring vibrations around 1600 cm^{-1} where **3** and **4** differ in relative intensities but where na also exhibits a strong band (Fig. 2b). Neat milling of 2:1 mixture of na and sub resulted in formation of **4** after ca. 30 minutes as indicated by the band at 1710 cm^{-1} (stretching of amide carbonyl group) but, unlike for X-ray diffraction, the intermediate **3** could not be clearly identified (Fig 2d).

The LAG cocrystallisation was performed using acetonitrile as the liquid additive. The transformation of the 2:1 mixture of na and sub was rapid, leading to the complete formation of **4** after 10 minutes which corresponds to 4 fold acceleration as compared to neat grinding. Nitrile stretching vibration is sensitive to its surroundings where hydrogen bonding with proton donors results in broadening of the $\text{C}\equiv\text{N}$ band and its shift to higher wavenumbers, which may be up to ca. 10 cm^{-1} in mixtures with e.g. water or methanol and even up to 30 cm^{-1} with very strong hydrogen bond donors such as HF.^[19]

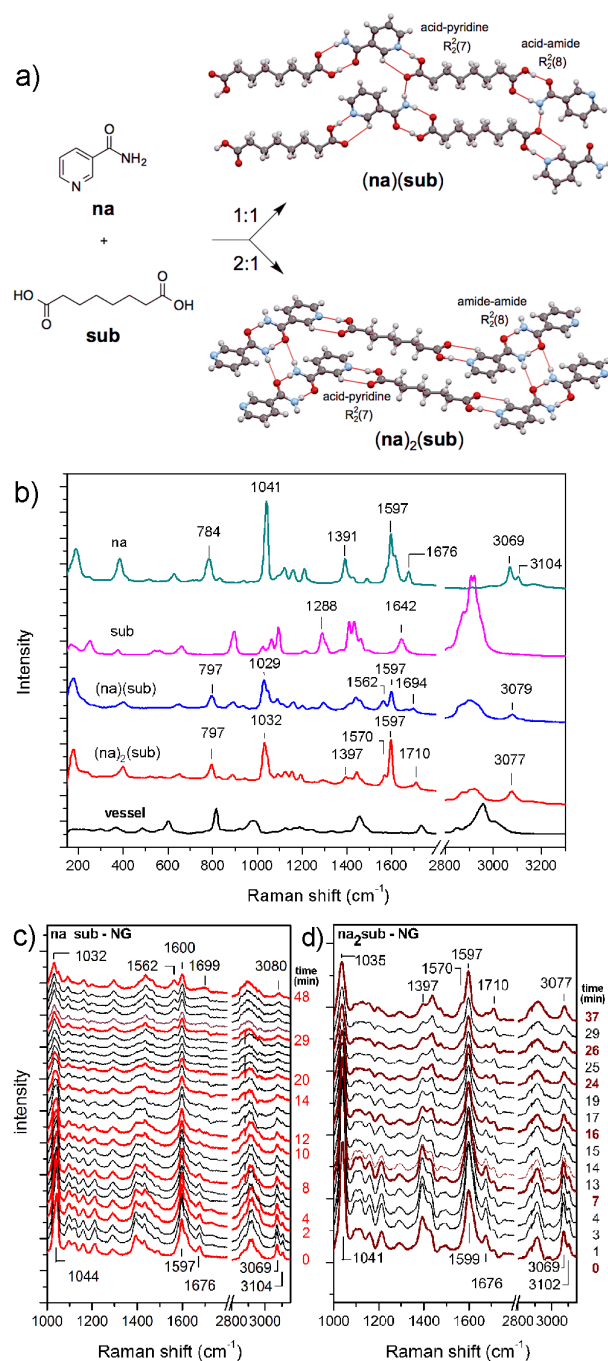


Figure 2. a) Cocrystal formation from nicotinamide and suberic acid where two cocrystals of different stoichiometries may be formed. b) Raman spectra of pure reaction participants. Time-resolved Raman spectra of neat grinding reactions using (c) 1:1 and (d) 2:1 reactant ratio of na:sub.

In our time-resolved LAG experiment (Fig. 3), the nitrile band (2253 cm^{-1}) is significantly broadened but remained at almost the same position as in liquid acetonitrile (2252 cm^{-1}) (Fig. S30). It also did not change its position and intensity during the whole milling. Thus, band broadening indicates acetonitrile participation in the LAG reaction mechanism but its extensive involvement in hydrogen bonding seems to be absent.

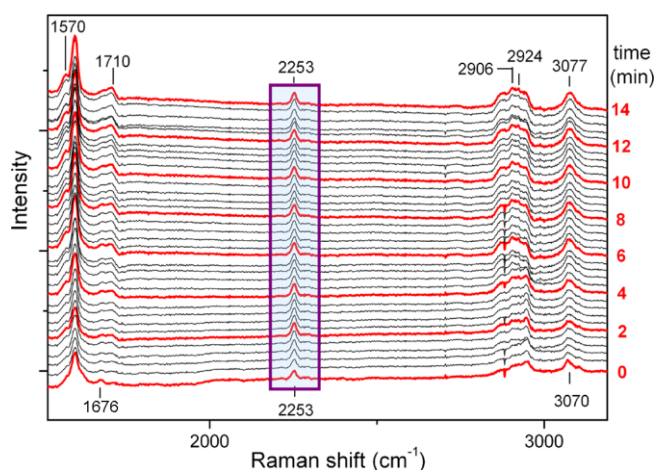


Figure 3. Time-resolved Raman spectra of LAG formation of the (na)₂(sub) cocrystal. The liquid acetonitrile band at 2253 cm⁻¹ is highlighted in the rectangle.

In summary, we have described the first real-time and *in situ* laboratory technique for monitoring the course of mechanochemical milling reactions by using Raman spectroscopy. When compared to the recent *in situ* X-ray diffraction method,^[13] *in situ* Raman scattering allows for a direct insight into the mechanochemical transformations at the molecular level and is thus sensitive for crystalline, amorphous and liquid reaction participants. The results presented here highlight the complexity of mechanochemical reactions and demonstrate the sensitivity of the reaction course on slight variations of reaction conditions. The technique was validated in neat and liquid-assisted mechanochemical formation of a coordination polymer and a model pharmaceutical cocrystal. Our findings strongly emphasise the need for *in situ* measurements to understand mechanistic aspects of milling reactions, since *ex situ* analysis may sometimes not accurately reflect the mechanism of mechanochemical reactions, as demonstrated here for the assembly of cadmium-based coordination polymers. We note that this *in situ* method, to the best of our knowledge, offers unique opportunities in studying the role of volatile liquid additives in milling reactions and should also be relevant for reactions where eutectic mixtures are formed resulting in partial or complete melting. The presented method is straightforward to implement in an every-day research laboratory and we expect it should gain widespread use in mechanistic investigations and optimisation of milling reactions.

Experimental Section

Raman scattering experiment was performed by using a portable Raman spectroscopy system assembled from the PD-LD LS2 laser source ($\lambda = 784$ nm) and Maya spectrometer (Ocean Optics) while Retsch MM200 or MM301 ball mills were operated at 30 Hz to conduct the milling reactions. The amplitude of the oscillations of the ball mill arms allows the probe to approach closely the horizontally oscillating vessel. The probe is optimally positioned below the vessel where more sample is being collected during milling. The vertical positioning of the Raman probe was adjusted using a precise movable stand such that the focus of the laser, which was at a distance of ~ 8 mm from the Raman probe, was inside the vessel. Possible laser-light-related heating of the sample is greatly or completely diminished by the constant oscillation of the vessel and mixing of the reaction mixture. The vessel contribution in the spectra was subtracted.

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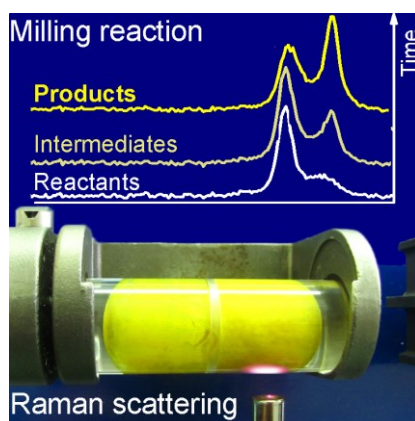
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Mechanisms of Mechanochemistry

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The obscurity of mechanochemical reaction mechanisms is challenged by developing a laboratory Raman spectroscopy technique for *in situ* and real time monitoring of milling reactions. The technique enabled following the course of mechanochemical transformations of coordination polymers and organic materials as well as the study of liquid additives.