Application of Low-Frequency Raman Scattering Spectroscopy to Probe in Situ Drug Solubilization in Milk during Digestion

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Supporting Information

ABSTRACT: We have recently shown that real-time monitoring of drug solubilization and changes to solid state of the drug during digestion of milk can be achieved using synchrotron small-angle X-ray scattering. A complementary laboratory-based method to explore such changes is low-frequency Raman spectroscopy, which has been increasingly used to characterize crystalline drugs and their polymorphs in powders and suspensions. This study investigates the use of this technique to monitor in situ drug solubilization in milk during the process of digestion, using a lipolysis model/flow-through configuration identical to that used previously for in situ synchrotron small-angle X-ray scattering studies. An antimalarial drug, ferroquine (SSR97193), was used as the model drug for this study. The Raman spectra were processed using multivariate analysis to extract the drug signals from the milk digestion background. The results showed disappearance of the ferroquine peaks in the low-frequency Raman region (<200 cm⁻¹) after approximately 15–20 min of digestion when milk fat was present in the system, which indicated drug solubilization and was in good agreement with the in situ small-angle X-ray scattering measurements. This proof-of-concept study therefore suggests that low-frequency Raman spectroscopy can be used to monitor drug solubilization in a complex digesting milk medium because of the unique vibrational modes of the drug crystal lattices.

Milk is a natural lipid-based formulation that has been shown to facilitate solubilization of a range of poorly water-soluble drugs during digestion and improve the oral bioavailability. Digestion of the triglycerides in lipid-based formulations (including milk) results in the release of diglycerides, monoglycerides, and fatty acids (the relative ratios of which depend on the extent of digestion) that form a progression of colloidal structures into which poorly water-soluble compounds in suspension can dissolve. This can circumvent the considerable risk of the drug precipitating out during digestion due to loss of solubilization capacity when the drug is predissolved in the lipid formulation. Techniques that enable in situ monitoring of drug solubilization and solid-state transformations (if any) during intestinal digestion are therefore valuable as these processes typically occur over a relatively short time scale. Separation of the digested lipid/drug samples by ultracentrifugation and analysis of the amount of drug precipitated in the denser pellet phase using high-performance liquid chromatography (HPLC) have traditionally been used to estimate the distribution of solubilized and nonsolubilized drug, based on the assumption that excess nonsolubilized drug was partitioned to the pellet phase after centrifugation. This approach is time-consuming and less well suited to providing in situ information on the solid-state form of drugs during digestion. Consequently, approaches were developed to enable in situ determination of drug precipitation and solubilization during digestion of lipid-based formulations using synchrotron small-angle X-ray scattering (SAXS). By tracking the increase or reduction of intensity or shifts in positions of characteristic diffraction peaks from the drugs to different scattering angles, the precipitation and solubilization or polymorphic transformations, respectively, were able to be quantified. However, this technique requires a high-intensity synchrotron source to provide the

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necessary sensitivity and time resolution to conduct such studies and thus is not amenable to routine use in formulation development and screening. Alternative approaches that are sensitive to the presence of crystalline material and have sufficient time resolution are therefore required.

Raman spectroscopy is a light-scattering technique that has been widely used to characterize the solid-state forms of active pharmaceutical ingredients (APIs) in the pharmaceutical industry because of its rapid analysis and nondestructive nature. It has also been used to monitor drug precipitation during in vitro lipolysis of a self-microemulsifying drug delivery system. Measurements typically acquire spectral information in the 200−1800 cm\(^{-1}\) fingerprint region to probe changes in molecular structures by characterizing the intramolecular vibrations that are sensitive to local functional group environments. Recent advancements in filter technologies such as volume Bragg gratings have allowed spectral acquisitions close to the laser frequency (within 10 cm\(^{-1}\)) in dispersive Raman systems. This low-frequency region (<200 cm\(^{-1}\)) can provide direct information on the intermolecular interactions within different solid-state forms of compounds. Intense Raman peaks related to vibrational modes of the crystal lattices (phonons) were observed in crystalline samples, while amorphous compounds that lack order typically give a broad peak. As a result, this technique has been increasingly used in recent years to detect solid-state transformation of drugs as powdered and suspension samples following heat treatment, quench cooling or grinding, to study the kinetics of amorphization of drugs during milling, and to identify different crystals in powder mixtures. However, to our knowledge, low-frequency Raman spectroscopy has not been used to track crystallinity and solubilization of poorly water-soluble drugs during digestion of lipid-based formulations. Herein, we explore the use of low-frequency Raman spectroscopy to probe the solubilization of a lipophilic drug in real time during digestion of milk under simulated small intestinal conditions. Ferroquine, a poorly water-soluble weakly basic antimalarial drug with \(pK_a\)s of \(\sim 7.0\) and 8.5 was used as the model drug in this study.

In vitro digestion of full cream bovine milk and buffer containing ferroquine was performed using the apparatus depicted in Figure 1 with experimental details being provided in the Supporting Information. Briefly, ferroquine (granules containing 50 wt % API provided by Sanoﬁ) was dispersed in milk or buffer as a suspension (186 mg granules in 2.75 mL of water and 17.5 mL of milk, which is approximately equivalent to a clinical dose in a glass of milk). The nutritional information on the bovine milk used in this study is summarized in Table S1 (Supporting Information). In an additional experiment, pretreatment of ferroquine with hydrochloric acid solution prior to the addition of milk was also carried out to mimic transiting through the gastric environment as was performed in the previous X-ray scattering studies. The samples were circulated through a standing quartz capillary (1.5 mm outer diameter) for 2 min prior to initiation of digestion. Digestion was initiated by injection of 2.25 mL of lipase suspension in tris buffer as described previously. Raman spectra were recorded over a 360 to 2030 cm\(^{-1}\) spectral window, with 5−7 cm\(^{-1}\) resolution, using a 785 nm excitation laser and a 135° backscattering geometry relative to the collective lens. Spectra were collected every 0.5 min for a total of 45 min (60 accumulations of 0.5 s exposure time). Further details of the experimental Raman setup and measurements are outlined in the Supporting Information.

The Raman spectrum of ferroquine exhibited strong phonon peaks at 34 and 46 cm\(^{-1}\) (Figure 2a) that are considered to be associated with lattice vibrations of highly ordered ferroquine crystals. These low-frequency peaks were still visually observable after dispersion in tris buffer and milk (Figure 2c−e) at a ferroquine concentration of about 4.6 mg mL\(^{-1}\).
with no formation of new peaks or peak shifts observed during stirring. This indicated that no solid-state transformations had occurred during dispersion of the solid drug in the buffer or milk media. The ability to detect low-frequency Raman spectra of drugs in suspension has also been demonstrated by Larkin et al. with indomethacin, albeit not to monitor a process or a suspension in a complex medium like milk.

Addition of lipase to start lipolysis of the fat component in milk caused a reduction in the intensities of the distinctive Raman scattering peaks of ferroquine, and the peaks subsequently disappeared during the course of digestion.

Figure 2. Low-frequency Raman spectra of (a) ferroquine (FQ) reference powder and the (b) milk background before and after digestion at pH 6.5 in the absence of ferroquine. Panels c–e show the Raman spectra of suspensions of ferroquine powder before and after digestion at pH 6.5 in (c) tris buffer, (d) milk, and (e) milk with ferroquine that has been pretreated with hydrochloric acid solution. The arrows point to the positions of the Raman peaks associated with crystalline ferroquine.
Figure 2d,e). However, in tris buffer that contained no digestible lipids, there was no change in the intensity of these Raman bands on the addition of lipase (Figure 2c). Disappearance of the peaks suggested loss of long-range order of the crystalline drug that could be attributed to drug solubilization and/or formation of an amorphous complex with the digested lipids. Inclusion of a gastric step where the ferroquine was exposed to hydrochloric acid prior to intestinal digestion also showed complete disappearance of the low-frequency Raman peaks after digestion. Less drug was initially present in crystalline form during dispersion after gastric pretreatment because of the greater solubility of basic ferroquine in the acidic environment (see Figure S1 in the Supporting Information). The presence of lipids and the process of digestion were therefore critical to the solubilization of ferroquine during digestion.

A closer examination of the Raman spectra was subsequently performed using principal component analysis (PCA) with Orange software version 3.16 to qualitatively interpret spectral variations between the samples. Linear baseline correction was applied to the Raman plots between $-250$ and $250 \text{ cm}^{-1}$, and the spectral region between $8$ and $200 \text{ cm}^{-1}$ was subjected to vector normalization prior to PCA. The plot of the first two principal components (which covered 95% of the sample variance with 56% in PC1) showed separate clusters for milk and milk/tris-containing ferroquine (FQ) due to spectral differences between the samples. Inspection of the loadings plot (shown in Figure 3b) revealed that Raman peaks were correlated with positive PC1, while no distinctive separation between ferroquine and the milk signals could be obtained from PC2 (Figure S2 in the Supporting Information). It was clear that all the samples containing crystalline ferroquine were clustered in positive

Figure 3. (a) Two-dimensional PCA scores plot for milk and milk/tris-containing ferroquine (FQ) based on analysis of the low-frequency Raman shift region from 8 to 200 cm$^{-1}$. Circled regions belong to clusters during dispersion, i.e., before lipase injection. (b) Corresponding loadings plot for PC1 (56% of sample variance) that described the ferroquine signals. (c) Plot of PC1 against dispersion (<0 min) and digestion (>0 min) time for all of the samples. (d) Percentage crystalline ferroquine in milk with gastric pretreatment during dispersion (<0 min) and digestion (>0 min) analyzed using SAXS. Values were determined from area under the diffraction peak associated with ferroquine at $q = 1.30 \text{ Å}^{-1}$, which was normalized to 100% of crystalline drug being present during dispersion. Lipase was injected at time = 0 min to initiate digestion. The experimental configuration for the SAXS runs was as previously described and used apparatus identical to the Raman scattering measurements (Figure 1).
PC1 values prior to digestion, and no changes were seen in tris/ferroquine samples with the progress of digestion (signifying no changes to the drug signal). Shifts in PC1 toward negative values were observed for the digesting milk/ferroquine samples. A plot of PC1 against dispersion (time < 0 min, suspension stirring before lipase injection) and digestion (time > 0 min, after addition of lipase) time in Figure 3c showed a gradual decrease in PC1 for the milk/ferroquine samples with and without gastric steps, which then plateaued around 15–20 min after lipase addition. Solubilization of ferroquine may therefore have reached completion within this time.

Although the fat content of the full-cream milk used for the Raman studies was slightly lower than that for the equivalent X-ray scattering studies (3.3% vs 3.8%, due to differences in the fat content of commercially available milk in New Zealand and Australia, respectively), the results obtained from the analysis of the low-frequency Raman spectra were in good agreement with the SAXS data where diffraction peaks associated with crystalline ferroquine also disappeared after about 15–20 min of digestion (Figure 3d). This signifies that 0.58 g of milk fat (17.5 mL of 3.3% milk fat) is sufficient to provide complete solubilization of 93 mg of ferroquine API (equivalent to 186 mg of ferroquine granules), as verified by the SAXS measurements. Another interesting observation was that analogous to differences between the ferroquine peak intensities between panels c and d of Figure 2, reduced PC1 values were also observed during dispersion in the milk/ferroquine sample that has been pretreated with hydrochloric acid. It is also worth noting that although the PC1 values in the tris/ferroquine sample stayed relatively constant throughout the digestion, a slight decrease in the PC1 value was observed after lipase injection, which could arise because of the slight dilution of the ferroquine as lipase suspension contributed ~10% of the total digestion volume.

Conclusions. We have demonstrated that low-frequency Raman spectroscopy can be used to monitor in situ solubilization of suspended ferroquine in milk during digestion. The presence of digestible fat in milk was necessary to achieve drug solubilization. Complete disappearance of the phonon peaks in the low-frequency Raman spectra was observed after about 15–20 min of digestion in milk when analyzed using multivariate analysis. This indicates complete drug solubilization, which is in agreement with previously obtained synchrotron small-angle X-ray scattering data. Further studies are now underway to investigate the wider utilization of this technique to monitor solubilization of other types of drugs in complex formulations (such as those containing excipients and bile salts) with simultaneous detection of solid-state transformations.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcllett.9b00654.

Experimental methods for flow-through in vitro lipolysis and low-frequency Raman spectroscopy, nutritional information for the milk used in the Raman scattering and SAXS measurements, loadings plot for PC2, and pH-dependent solubility of ferroquine (PDF)

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Notes

The authors declare the following competing financial interest(s): Stephane Belles is an employee of Sanofi, which owns the model drug ferroquine used in these studies.

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