Experimental Study on the Raman Spectra of Imine Emulsification with Chemometrics

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Abstract: In this study, we aimed to investigate imine emulsification using Raman spectroscopy with chemometrics. The imine emulsification samples were obtained by mixing aldehydes and amines in methanol and aqueous methanol. The Raman spectra of the samples were measured over time between 400 and 2300 cm⁻¹ every 40 s using a Raman spectrometer. The obtained spectra were regarded as a dataset matrix. A multivariate curve resolution with alternating least squares was applied to the dataset. A multivariate analysis based on the Raman spectrum revealed that raw materials, emulsions, and products were decomposed when the water-rich samples were emulsified. Additionally, we evaluated the kinetics of the synthesis. The effect of water content on emulsification was investigated using Raman spectroscopy. The molecular dynamics of the co-solvent model were also investigated. The phase-layer construction was consistent with the phase transition in the water-methanol imine samples.

Key words: Raman spectroscopy, process monitoring, imine synthesis

1 Introduction

Two liquids can form different types of emulsions, such as water-in-oil and oil-in-water emulsions. By controlling the interface between water and oil, useful applications can be found in various scientific fields. In the field of pharmaceuticals, external preparations, liposome preparations, etc., can be prepared by combining with a surfactant. In addition, emulsions with surfactants are available as targeting systems for drug delivery systems. Brenhard et al.¹ reported albumin nanoparticles appropriate for future drug-targeting studies. However, only a few studies have evaluated the preparation of liposomes or emulsions over time. We observed novel results in assessing temporal changes in the preparation of imine emulsions as model compounds. We believe that performing synthesis and emulsification (i.e., a single-step formulation) would lead to a green chemistry era.

Process analytical technology (PAT) has already been incorporated into the manufacturing process of pharmaceutical products. The U.S. Food and Drug Administration (FDA) recommends PAT using the Raman², infrared, near-infrared, and terahertz spectra during the pharmaceutical formulation process³. Noncontact spectrophotometry is ideal for quality preservation. In addition to the formulation process, examples of spectrophotometry applications that maintain the quality of products in biology have been reported⁴. An increasing number of applications to flow synthesis have also been reported recently. Wensink et al.⁵ developed a microfluidic chip for nuclear magnetic resonance (NMR) spectroscopy on samples whose volumes were less than 1 µL. Fath et al.⁶ reported on PAT for imine and lithiated intermediate synthesis using Fourier-transform infrared spectroscopy (FT-IR) flow and multivariate analyses.

Because Raman spectroscopy is both contact-free and nondestructive, samples can be measured without pretreatment. This technique enables in-line and on-line monitoring of the quality of organic synthesis process. Many studies conducted quantification and qualification analyses for a wide range of samples⁷. This method is also used to analyze industrial products, foods, and pharmaceuticals. In the case of Raman spectra that are complicated owing to the overlapping bands, multivariate analysis may be advantageous. Adopting multivariate spectroscopy has popularized the use of chemometrics to extract qualitative information from data⁸.

In our previous study⁹, we predicted the effect of pseudo-polyorphism on the thermal analysis of theophylline tablets using Raman spectroscopy using the multivariate analysis. Another study⁹ reported that multivariate curve resolution with alternating least squares (MCR-ALS) is beneficial for the quantification analysis of cocrystal process.

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monitoring based on powder X-ray diffractograms. In this study, we applied a combination of Raman spectra and multivariate approaches to explain imine emulsions thoroughly. The effect of the water content on the imine emulsion was elucidated using Raman spectroscopy and MCR-ALS. Imine synthesis is usually achieved using methanol. Simulated models of the solvents and products were calculated using a molecular dynamic process. The driving force for constructing several phases was studied using molecular interactions between the solvent and the product.

2 Materials and Methods

2.1 Materials

Benzaldehyde (compound 2) and super-dehydrated methanol were purchased from Fujifilm Wako Pure Chemical Industries, Ltd. (Japan). Benzylamine (compound 1) was purchased from Tokyo Chemical Industry, Japan.

2.2 Methods

2.2.1 Sample preparations

N-benzylidene benzyl amide (compound 3) was prepared by mixing 4 mmol of compound 1 and 4 mmol of compound 2 with a methanol and water mixture solvent with a concentration of 4 mol/L. The water concentration was varied from 0 to 15%. A magnetic stirrer (CC-301, AS ONE Co., Japan) was used for preparing the synthesis, with the rotation power set to level 8 at room temperature.

2.2.2 Raman spectroscopy

Using a Raman spectrometer (Mira DS, Metrohm Japan Ltd.), the Raman spectra of samples were measured in the range of 400–2300 cm⁻¹ with a resolution between 8 and 10 cm⁻¹.

2.2.3 Multivariate analysis

Raman spectra were analyzed using MCR-ALS. The Raman spectra dataset can be arranged in a data matrix \( D_{(r \times c)} \), with the spectra as \( r \) rows and \( c \) columns, which are the measured values at the respective layers.

The MCR decomposition of dataset \( D \) was performed according as follows:

\[
D_{(r \times c)} = C_{(r \times n)} S_{(n \times c)} T + E_{(r \times c)},
\]

where matrix \( C_{(r \times n)} \) is the concentration profile that describes the individual contributions of \( n \) species involved in \( D \). Matrix \( S_{(n \times c)} T \) is the contribution of \( n \) species in the \( c \) columns of the Raman spectra. \( E_{(r \times c)} \) is the residual matrix and considered to be noise data during model construction. In addition, it can be eliminated from the Raman spectra when two components are considered. Only positive \( S \) and \( C \) values were observed. The calculations were performed using Unscrambler® X, version 10.5.1, from CAMO Software (Trondheim, Norway).

2.2.4 Raman spectra simulation using the density-function theory

Density functional theory (DFT) was applied to investigate the Raman spectra of the products and materials using the Dmol® package from BIOVIA Materials Studio 2019. Benzaldehyde, benzylamine, and N-benzylidene benzyl amide were calculated for optimization, frequency, and Raman intensities: functional: LDA-PWC; noncharge; Core Treatment: All electrons; Basis set: DND; Basis file 3.5; SCF tolerance: 0.00001; without a solvent model under vacuum conditions. The calculated Raman spectra were normalized by setting the highest intensity to 100% in the range of 0–4000 cm⁻¹.

2.2.5 Molecular dynamics (MD) simulation

MD simulations were performed using the Forcite package from BIOVIA Materials Studio 2019 (ver. 19.1.0.2353). Two amorphous cells \((a = b = c = 18.1 \text{ Å})\) were prepared in water:MeOH-N-benzylidene benzyl amide ratios of 0:62:8 and 16:53:8, respectively. The MD was calculated using COMPASS II force field®. The ensemble was set as the NVE. The temperature, time step, and total simulation time were 298 K, 1.0 fs, and 500.0 ps, respectively. An atom-based summation method was used for electrostatic and Van der Waals forces.

3 Results and Discussion

3.1 Raman spectroscopy for imine emulsion

Figure 1 shows the imines synthesized by mixing compounds 1 and 2 using a magnetic stirrer. Figure 2 shows the temporal evolution of the Raman spectra during imine synthesis. For a sample containing 0% water initially, specific Raman spectra peaks were observed at 1704, 1647, 1597, 1455, 1206, 1169, 1029, 1004, 852, 830, 796, 752, 653, 620, and 441 cm⁻¹. After the synthesis, the peak intensity at 1704 cm⁻¹ due to \( \nu(C=O) \) decreased over time, while the peak intensity at 1645 cm⁻¹ due to \( \nu(C=N) \) increased. Compounds 1 and 2 were synthesized as compound 3. The peaks at 1597 and 1206 cm⁻¹ shifted to 1604 and 1223 cm⁻¹, respectively. The sample with 10% water content exhibited broad pronounced peaks from 1270 to 2300 cm⁻¹ at 320 s. Samples containing 15% water showed comparable broad peaks.

After synthesis, when the magnetic stirrer of the samples with 10% and 15% water content was turned off and the

Fig. 1 Imine synthesis scheme and molecular structure of 1) benzylamine, 2) benzaldehyde, and 3) N-benzylidene benzyl amide.
samples were allowed to stand still for 30 min, the phase was separated into two layers. The broad peak indicates the Tyndall effect in the phase-separated samples. Because the water could not be completely dissolved during the synthesis, it was separated and stirred in the mixed solvent to form an emulsion. The water provides a baseline for the Raman spectra. Lyndgaard et al.\textsuperscript{11} performed a quantification analysis of pharmaceutical ingredients using Raman spectroscopy with MCR-ALS. Treating the Raman spectra as a dataset, MCR-ALS was applied to investigate the changes in the concentration and kinetics of the products and raw materials.

3.2 MCR-ALS analysis based on the Raman spectra

Figure 3 shows the MCR-ALS sources for (A) the Raman spectra dataset with three components, (B) the obtained material spectra, and (C) calculations based on the DFT for the material spectra. The profiles of Sources 1 (S1), 2 (S2), and 3 (S3) have specific Raman intensity peaks owing to the raw material mixture spectra, scattering from the Tyndall-effect spectra, and the three spectra produced. DFT is effective for the spectra assignment.

\[\text{Fig. 2 Effect of water/MeOH solvent on the imine synthesis of benzaldehyde and benzylamine. (A) Water 0\%(v/v) MeOH solvent, (B) water 5\%(v/v) MeOH solvent, (C) water 10\%(v/v) MeOH solvent, and (D) water 15\%(v/v) MeOH solvent.}\]

\[\text{Fig. 3 Sources of MCR-ALS based on the acquired Raman spectra dataset of the synthesized samples. (A) Sources, (B) Raman spectra of materials with MeOH, and (C) simulated spectra of bulk materials using DFT calculations.}\]
Krishnakumar et al. calculated the Raman spectra of 2-(methylthio)benzonitrile using DFT\(^{12}\). The obtained spectra were interpreted using coordinate analysis following structural optimization based on DFT. In this study, DFT was applied to a single molecule of compounds 1, 2, and 3 in vacuum, and the calculations were performed using LDA-PWE. DFT results, which were comparable to those of spectra 1, 2, and 3, were useful for assigning sources.

Figure 4 presents the results of MCR-ALS calculation as a concentration vs. time based on the Raman spectra. Based on the values assigned in the previous section, the temporal variation in the sample concentration provided the scattering intensities of the product, emulsion, and unreacted raw material. The samples with 0 and 5\% water content that did not emulsify, exhibited extremely low S2 values. The material mixture spectrum of S1 gradually increased over time in the samples with a water content of 0 and 5\%. S3, which was produced using compound 3, increased over time.

Conversely, the samples containing 10 and 15\% water with laser scattering based on emulsification showed a significant change in the value of S2. The spectral changes associated with the phase formation were 280 and 120 s for the samples with 10 and 15\% water content, respectively. The time change of S3 revealed that emulsion formation was stabilized in 180 s, and the concentration of the synthesized compound 3 decreased in the sample with 15\% water content. After the emulsion was formed, the values of S3 for the samples with 10 and 15\% water content increased. These time differences were attributed to the solubility in the solvent, depending on the amount of water. At the time of 480 s, the percentages of S3 for samples A and B were 80.3 and 82\%, respectively.

After a significant change in the value of S2, we inferred the following from the time variation of the decomposed concentration: (1) an increased amount of compound 3 was produced, which became insoluble in the solvent, (2) a soluble phase was formed, and (3) the two layers were mixed using a magnetic stirrer to form an emulsion. The Raman spectrum, combined with MCR-ALS, makes evaluating an emulsion in imine synthesis possible. Wang et al.\(^{13}\) quantified \(\alpha\)-tocopherol using Raman spectroscopy. Its distribution in oil-in-water emulsions was visualized when it was stabilized by a whey protein isolate.

The kinetics of imine synthesis was evaluated. The half time of S1, which is the amount of material, was calculated for the samples with 0 and 5\% water content using the Microsoft Excel solver as follows:

\[
y = y_0 (1-e^{-kt}) + y_\infty,
\]

where \(y\), \(y_0\), \(y_\infty\), \(k\), and \(t\) are the current, initial, and final concentrations of S1, a constant, and time (s), respectively. The half times of the samples with 0 and 5\% water content were 28.8 \(\pm\) 2.1 and 26.9 \(\pm\) 2.1 s, respectively. Consequently, the samples with 5\% water content were faster than the samples with 0\% water content.

### 3.3 Effects of water on the phase separation

Figure 5 shows photographs of the samples that were allowed to stand still for at least 30 min after the synthesis process. The samples containing 10 and 15\% water were phase-separated. The coalescence of samples C and D was formed within 30 min. The phase-separated sample was stable for at least 1 h. Phase separation perhaps occurred by the interaction among compound 3, water, and metha-
MD simulations were performed to analyze the layer separation mechanism. The MD simulation model was generated as a model of a sample with 0% water content with a mixture of compound 3 and methanol, randomly placed in cells in the form of a cube with sides of 18 Å. A model of compound 3 in a methanol solvent containing 15% water had a water:MeOH:compound 3 = 16:53:8 ratio of water, compound 3, and methanol. The ratio of compound 3 to methanol was set at water:MeOH:compound 3 = 0:8:62, assuming a concentration of 4 M. A dynamic calculation for 500 ps was applied to both models.

The MD simulation results are shown in Fig. 6. Figure 6A shows the supercell of simulated compound 3 and the methanol model. In the figure, it is a water-less model, and a homogeneous molecular distributed state is indicated. Methanol and compound 3 were mixed completely owing to their interactions. In Fig. 6B, the model constructed two layers: a mixture of compound 3-methanol, and methanol-water layers. Consequently, a sample containing 15% water separates into two layers. Affinities were found between the methanol and water.

4 Conclusion

The effects of water on imine emulsification were studied based on the Raman spectra and MCR-ALS. MCR-ALS was applied to the Raman spectra, resulting in a successful decomposition. DFT was used for source assignments. The spectral dataset was decomposed into products, scattering, and materials. Both the samples with 0 and 5% water content were found to possess comparable values for half time of the materials. Samples containing over 10% water (v/v) methanol solvent showed phase separation and formed an emulsion. Emulsion evaluation using Raman spectroscopy and the Tyndall-effect were useful in monitoring the effect on the sample conditions and reaction rate. Molecular interactions for the construction of emulsions were investigated using MD simulations. Consequently, when the methanol solvent contained 10% or more water, imine dissolution was limited, and phase separation occurred. The results of this study not only demonstrated the effects of water on imine emulsification but also provided a basic approach to drug development for synthetic process monitoring. Process monitoring using the Raman spectra in the pharmaceutical manufacturing process is significantly important in guaranteeing the quality of final products. Although the number of reports
on flow-based synthesis methods has been increasing in recent years, the combination of Raman spectroscopy and chemometrics is considered as an advanced technology that supports the formation of stable products in tubes. In a future study, we would like to investigate emulsification with surfactants and monitor flow synthesis process using Raman spectroscopy with chemometrics.

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Conflict of Interest
The authors declare no conflict of interest.

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