Development of Universal Formulation with Superior Re-dispersion Using Nanocrystal Approach with Simultaneous Identification of API Physicochemical Properties

Hiroyuki Fujii and Satoru Watano*

Department of Chemical Engineering, Graduate School of Engineering, Osaka Prefecture University; 1–1 Naka-ku Gakuen-cho, Sakai 599–8531, Japan.

Received February 3, 2019; accepted July 1, 2019

Universal nanocrystal formulation which can be applied to water-insoluble compounds was proposed and the criteria of its physicochemical properties as an active pharmaceutical ingredients (API) were investigated. Nanocrystal suspension was prepared by a wet-beads milling method. An acceptable Critical Quality Attributes (CQA) of nanocrystal suspension was defined by Z-average less than 500 nm and Polydispersity index (PDI) less than 0.3. Screening studies of dispersing and wetting agents were conducted using three model compounds in different pH, melting points, etc., to find universal nanocrystal formulation. The effect of four structurally different polymer species (hydroxypropyl cellulose (HPC), hydroxypropyl methylcellulose (HPMC), polyvinylpyrrolidone (PVP) and polyvinyl alcohol (PVA)) and their different grades or five different surfactants (docusate sodium (DOSS), sodium lauryl sulfate (SLS), cetetyl trimethyl ammonium bromide (CTAB), polysolbate80 (PS80), and polyoxyethylene castor oil (CO-35)) were studied on the re-dispersion stability. It was found that the combination of 4% (w/v) HPC-SSL and 0.2% (w/v) DOSS was the most robust nanocrystal formulation owing to Z-average less than 200 nm and good re-dispersion stability without aggregates at pH 1.2 and pH 6.8. API physicochemical properties were also identified using ten water-insoluble compounds. Consequently, it was found that solubility (water, pH 1.2 and pH 6.8), molecular weight, hydrogen bonding acceptor and the ratio of logD∞ to CLogP were critical factors.

Key words nanocrystal; wet beads milling; re-dispersion stability; dispersing agent; wetting agent

Introduction

Recently, new chemical entries which show highly water-insoluble characteristics have increased because of the shift in target therapeutic area from lifestyle-related diseases to central nervous system (CNS) or oncology in the pharmaceutical industries. Ninety percent of new chemical entries are classified as highly water-insoluble compounds, and seventy percent of them have been terminated from further pharmaceutical development since it is difficult to demonstrate the efficacy and safety in the preclinical stage due to the low bioavailability caused by the poor solubility. Therefore, highly water-insoluble compounds are considered to have a high risk in pharmaceutical development. In order to increase the probability of success, solubilized formulation technology is expected to improve the bioavailability of highly water-insoluble compound classified into the biopharmaceutical classification (BCS) class II or class IV. Representative solubilized formulation technology implies crystal engineering, prodrug, self-micro emulsifying drug delivery system (SMEDDS), cyclodextrin inclusion, solid dispersion, liposome and micellar system. Particle size reduction of active pharmaceutical ingredients (API) is also effective as solubilized formulation technology. As for the manufacturing process of particle size reduction, there are two approaches; bottom up or top down. The bottom-up approach is a method that obtains ultra-fine particle from 1 to 100 nm by nucleation which generates the particle from molecule level in gas or liquid phases. On the other hand, the top-down approach is a method that obtains submicron particle from 100 to 100 µm by pulverizing with high mechanical energy such as power mill, pin mill, jet mill, microfluidizer, beads mill and high-pressure homogenizer. Nanocrystal has a significant increase in dissolution rate or solubility as compared with un-milled API. The dissolution rate is described by Nernst–Brunner/Noyes–Whitney equation as follows:

$$\frac{dC}{dt} = k \times A(C_s - C)$$

where $C, A, C_s$ and $k$ mean the API concentration in solution at time $t$, surface area of API, saturated solution and apparent dissolution rate constant, respectively. Apparent dissolution rate constant $k$ varies by solvent volume, temperature, solvent viscosity and stirring condition. This equation shows that the increase in surface area due to the particle size reduction leads to the improvement of dissolution rate. The estimation of the solubility after pulverization is described by Freundlich-Ostwald equation as follows:

$$S = C_{ss} \exp \left( \frac{2\gamma M}{\rho R T} \right)$$

where $S, C_{ss}, \gamma, M, \rho, R$ and $T$ show saturated solubility, the solubility of API having radius $\infty$, solid-liquid surface tension, molecular weight, radius of API, density, gas constant and absolute temperature, respectively. This equation implies that solubility increase depends on particle size reduction. However, particle size less than 100 nm is required to improve the solubility.
To achieve the highest increase in dissolution rate and solubility, it is important to avoid the aggregation of nanocrystals. Even though primary particle size reduction is realized, submicron particle still tends to aggregate due to van der Waals forces. It is common practice to add anionic or cationic charge surfactants on the particle surface. The value of particle surface charge indicates the stability of nanocrystal suspensions at the macroscopic level. A minimum zeta potential of \( \pm 30 \text{ mV} \) is required for electrostatically stabilized nanocrystal suspensions. It is also reported that nanocrystal suspension can be stabilized by polymer modification on the surface of nanocrystal to work as static repulsion force by the interaction between polymer chains. A minimum of \( \pm 20 \text{ mV} \) zeta potential is also necessary for steric stabilization. Although zeta potential provides an indication on nanocrystal stability, it does not reflect entire picture. The selection of appropriate stabilizer composition is a crucial step in achieving stable nanocrystal suspensions. Wu et al. reported that most of nanocrystal suspensions were generated in aqueous medium with sodium dodecyl sulfate (SDS), sodium lauryl sulfate (SLS), lecithin and docucate sodium, Pluronic surfactants, tween 80, polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), hydroxypropyl cellulose (HPC) and hydroxypropyl methylcellulose (HPMC) based on 76 published studies. Nakach et al. investigated that an optimum wetting and dispersing agent were selected for preparing nanocrystal suspensions of model compound using a combination of empirical and colloidal-interfacial fundamental approach. The PVP/SDS mixture was selected by excluding the sample of a particle diameter greater than 500 nm, zeta potential less than 15 mV and a viscosity higher than 10 mPa·s. George and Ghosh investigated the optimization of nanocrystal suspension using media milling approach with 6 model compounds. The HPMC 3 cps/SDS mixture showed a particle size less than 500 nm, zeta potential higher than \( -30 \text{ mV} \) and good stability during 6 weeks. Ghosh et al. developed a promising nanocrystal suspension formulation with Vitamin E TPGS, which produced better results compared to non-micronized formulation during in vitro dissolution study and in vivo dog pharmacokinetics (PK) study. The optimization of the formulation with HPMC 3 cps resulted in inhibiting crystal growth during stability as compared to other stabilizers such as PVP K30, Pluronic or SLS. Some researchers reported similar studies of nanocrystal suspension development with their discovery pipeline or model compounds to demonstrate improvements in in-vivo parameters. Thus, it is clear that different APIs require different stabilizers. Lack of a systematic understanding on selection criteria of stabilizers implies the use of the trial-and-error method by academics and the pharmaceutical industry. Furthermore, in oral administration, nanocrystals are exposed to gastric acid and digestive fluid in the digestive tract until absorption. Nevertheless, previous studies were not evaluated dispersion stability against simulated gastric conditions; therefore, they were insufficient to find optimized stabilizer.

Both the physical properties of the stabilizers and surface properties of the API contributes towards the mechanism of stabilization. Choi et al. studied HPC and PVP as stabilizers for seven drug nanocrystals produced by the wet-milling technique. The interaction characteristics between stabilizers and drugs are a function of various parameters including surface energy and the existence of functional groups. Eerdenbrugh et al. conducted an expand study using 13 stabilizers at 3 different concentrations to stabilize 9 drug compounds by wet milling technique. No correlation was observed between drug physicochemical properties and nanocrystal suspensions formation success rate. It was demonstrated that the surface hydrophobicity of the drug candidates was the driving force for nanoparticles agglomeration. George and Ghosh concluded that the stabilizer and drug interaction seemed to be a stronger factor than surface charge itself. API with low enthalpy values were observed to be poor candidates for formulation as nanocrystal suspension irrespective of the stabilizer used. There is not enough literature in terms of the mechanism of stabilization with physicochemical properties of API. Therefore, the criteria for physicochemical properties of API appropriate for nanocrystal technology are not clear.

In this study, universal nanocrystal formulation which can
be applied to water-insoluble compounds was proposed, and the criteria of its API physicochemical properties were investigated. Nanocrystal suspension was prepared by a wet-beads milling method. Nifedipine, fenofibrate and indomethacin classified in BCS class II which have different pK_{a} were selected as the model compounds to propose universal nanocrystal suspension. The screening of polymer and surfactants was conducted to find robust nanocrystal suspension formulation which did not aggregate against 1st fluid (pH 1.2) and 2nd fluid (pH 6.8) for dissolution test of Japanese Pharmacopeia (JP1 and JP2). API physicochemical properties were identified using ten water-insoluble compounds which have different properties.

### Experimental

#### Materials

Three highly water-insoluble BCS class II classified compounds, namely, nifedipine, which is a weak base (Daito pharmaceutical, Japan), Fenofibrate, a neutral compound (Moehs coprima, Spain), Indomethacin, which is an acid-base compound (Sogo pharmaceutical, Japan) were used as model API for screening studies. The physicochemical properties and morphology of the model APIs are indicated in Table 1 and Fig. 1, respectively. Other APIs were also used as model compound classified in BCS class II. Cilostazol was purchased from Daito Pharmaceutical (Japan). Phenytoin, bezafibrate, itraconazole, piroxicam, dipyridamole, and ketoprofen were purchased from Wako (Japan). The dispersing agents and wetting agents used in this study are listed in Tables 2, 3, respectively. These tables were summarized from each vendor’s

#### Table 1. Physicochemical Properties of Drug Substances

|                  | Nifedipine | Fenofibrate | Indomethacin |
|------------------|------------|-------------|--------------|
| Chemical structure | ![Structure](image1) | ![Structure](image2) | ![Structure](image3) |
| Molecular weight, Mw [g/mol] | 346.33 | 360.83 | 357.79 |
| Melting point, Mf [°C] | 171 | 78 | 159 |
| pK_{a} | Weak base | Neutral | Acid |
| CLogP | 3.31 | 5.23 | 4.18 |
| Solubility [µg/mL] | JP1 (pH1.2) | 7.7 | 0.3 | 0.7 |
| JP2 (pH6.8) | 7.3 | 0.3 | 762 |
| Particle size [µm] | D_{10} | 2.1 | 1.7 | 4.3 |
| D_{50} | 13 | 5.6 | 20.7 |
| D_{90} | 45 | 11.7 | 75.4 |

(a) Refer to L.Z. Benet et al., The AAPS Journal, Vol. 13, No. 4, December 2011. (b) Measured by modulated DSC. (c) 37°C2h. (d) Measured by laser diffraction volume-based particle size distribution.

#### Table 2. Dispersing Agents Used for Screening Study

| Polymer                  | Grade | Viscosity    | Chemical structure | Vendor                                |
|--------------------------|-------|--------------|--------------------|---------------------------------------|
| Hydroxypropyl Cellulose (HPC) | SSL   | 2.0-2.9 (°)  | ![Structure](image4) | Nippon Soda Co., Ltd. (Japan)         |
|                          | L     | 6.0-10.0 (°) | ![Structure](image5) |                                        |
| Hydroxypropyl/methyl Cellulose (HPMC) | TC-5E | 3 (°)        | ![Structure](image6) | Shin-etsu chemical Co., Ltd. (Japan)  |
|                          | TC-5R | 6 (°)        | ![Structure](image7) |                                        |
| Polyvinylpyrrolidone (PVP) | K30   | 5.5-8.5 (°)  | ![Structure](image8) | BASF (Japan)                          |
|                          | K90   | 300-700 (°)  | ![Structure](image9) |                                        |
| Polyvinyl Alcohol (PVA)  | EG-03P| 3.0-3.7 (°)  | ![Structure](image10) | The Nippon Synthetic Chemical Industry Co., Ltd. (Japan) |
|                          | EG-05P| 4.8-5.8 (°)  | ![Structure](image11) |                                        |
|                          | EG-18P| 16.0-20.0 (°)| ![Structure](image12) |                                        |
|                          | L-3266| 2.3-2.7 (°)  | ![Structure](image13) |                                        |

(a) 2 wt% aqueous solution at 20°C. (b) 4 wt% aqueous solution at 20°C. (c) 10 wt% aqueous solution at 20°C.
technical catalogs. Dispersing agents and wetting agents were selected from common polymer and surfactant in the pharmaceutical industry. Other reagents for quality testing were purchased from Wako (Japan).

**Methods**

**Suspension Preparation for Dispersing Agent and Wetting Agent Screening Studies**

The nanocrystal suspensions were prepared by a nano pulverizer (NP-100, THINKEY, Japan), which is a planetary type centrifugal pulverizer, using 0.1 mm zirconium beads (YTZ® ball, Tosoh, Japan) as the milling media. In the case of dispersing agent screening, each API suspensions were containing 20% (w/v) API with 0.2% (w/v) DOSS and polymer solution at constant viscosity of 4% (w/v) HPC-SSL, 1.4% (w/v) HPC-L, 3% (w/v) HPMC TC-5E, 1.9% (w/v) HPMC TC-5R, 5% (w/v) PVA EG-03, 4% (w/v) PVA EG-05, 2% (w/v) PVA EG-18, 8.7% (w/v) PVA L-3266, 9.5% (w/v) PVP K30 or 1.76% (w/v) PVP K90, respectively. Target viscosity was $5.4 \pm 0.2$ mPa·s which was same as 4% (w/v) HPC-SSL. In the case of wetting agent screening, each API suspensions were containing 20% (w/v) API with 4% (w/v) HPC-SSL and 0.2% (w/v) DOSS, 0.2% (w/v) SLS, 0.2% (w/v) CTAB, 0.2% (w/v) PS80 or 0.2% (w/v) CO-35, respectively. The milling was conducted at 1700 rpm, under 6 min operation time and chilling temperature ($-20°C$).

**Suspension Preparation for API Physicochemical Property Correlation Study**

The nanocrystal suspensions were prepared by the nano pulverizer using 0.1 mm zirconium beads. In this study, phenytoin, cilostazol, itraconazole, bezafibrate, piroxicam, dipyridamole and ketoprofen were selected as an additional model compound. Each suspension was containing 20% (w/v) API with identical composition of 4% (w/v) HPC-SSL and 0.2% (w/v) DOSS. The milling was conducted under the conditions of rotating speed; 1700 rpm, operation time; 6 min and chilling temperature; $-20°C$.

**Viscosity Measurement**

A vibration-type viscometer (Viscomate model VM-10A, SEKONIC, Japan) was used to measure the viscosity of polymer solution at room temperature using a grass bottle.

**Particle Size and Zeta Potential Measurement for Nanocrystal Suspension**

Particle size distribution and zeta potential of nanocrystal suspension were measured by a particle size and a zeta potential measurement apparatus (Zetasizer nano ZS, Malvern Instruments, Japan) using a dynamic light scattering method by intensity. Particle size distribution showed Z-average and polydispersity index (PDI). The measurement of particle size and zeta potential were carried out using a clear disposable zeta cell and calculated from the average of 10 measurements. The measurement samples were prepared as follows: nanocrystal suspension was weighed 69 mg and fed into a 100 mL volumetric flask. Nanocrystal suspension was then diluted with ultra-pure water (water), JP1 and JP2. The diluted nanocrystal suspension was obtained after gently shaking without sonication. The aggregation of nanocrystal in vitro was evaluated based on the stability against different salt concentrations and pH.

**Powder X-Ray Diffractometer (PXRD)**

A powder X-Ray diffractometer (PXRD) was used to measure the crystallinity of APIs before and after the wet-beads milling process. The measurement sample of nanocrystal API was obtained by drying the nanocrystal suspension on the glass plate at room temperature overnight. The X-ray diffraction pattern was measured using an X-ray diffraction apparatus (RINT 2500, Rigaku, Japan).

**Modulated Differential Scanning Calorimetry (DSC)**

A differential scanning calorimeter (Discovery DSC™, TA Instruments, U.S.A.) was used to measure the melting point of each APIs and nanocrystal APIs. Approximately 2 mg of API was sealed in an aluminum pan and used for measurement. Also, 10 µL of nanocrystal suspension as equivalent 2 mg of API was filled, dried at room temperature, sealed in an alu-
The success rate of nanocrystal suspension was reported and investigated. It is not necessary to misunderstand the effect of polymer by considering viscosity. Therefore, the wet-beads milling was conducted using three different APIs to investigate the impact, friction, and shear stress generated between zirconium beads mostly convert to heat energy. Although fenofibrate has a low melting point of around 78°C, there was a potential risk that the heat energy might impede fenofibrate during milling. This risk was overcome by chilling step around –20°C during the milling process. As a result of wet beads milling, fenofibrate nanocrystal with a Z-average less than 200 nm was obtained using HPC-SSL or HPMC TC-5E. Especially, the nanocrystal with HPMC TC-5E was the PDI less than 0.2. On the other hand, the nanocrystal suspension with PVP EG-18, PVA L-3266 or PVP K90 had a Z-average more than 300 nm and PDI more than 0.3, implying that these dispersing agents did not meet the acceptable CQA. Therefore, the best-suited polymer for fenofibrate was HPMC TC-5E, followed in order by HPC-SSL and PVP K30, and PVA EG-03.

It was found that all indomethacin nanocrystal suspensions met the acceptable CQA. The Z-average in the case of indomethacin nanocrystals was less than 300 nm. Particularly, in the nanocrystal with HPC-SSL, HPC-L, PVP K30 and PVP K90, a Z-average less than 200 nm was observed. Also, the PDI of the nanocrystal with PVP K30 and PVP K90 was smaller than the PDI of HPC-SSL and HPC-L. Thus PVP K30 or PVP K90 were best-suited polymer for indomethacin, however even in the case of indomethacin one of the best-suited polymer was HPC-SSL.

The possible reasons for HPC-SSL to qualify as the better polymer for wet-beads milling process were investigated from physicochemical properties of API. The bonding force between API might cause aggregation during the milling process. Drag force of bonding is described by a function of surface tension so that lower surface tension leads to low drag force. That means lower surface tension has certain advantages to prevent aggregation by the steric effect of absorbed polymer on API surface. If the polymer does not absorb on API surface, the steric hindrance effect does not work to prevent the aggregation. Surface tensions of each polymer solutions were already reported for water-72 mN/m, HPC-44 mN/m, HPC-L 22 mN/m, HPC-SSL 19 mN/m, HPMC TC-5R 28 mN/m, and PVA L-3266 27 mN/m. Thus, lower surface tension has certain advantages to prevent aggregation by the steric effect of absorbed polymer on API surface.

Table 4. Z-Average, PDI and Zeta Potential under Various Kinds of Dispersing Agents with Nifedipine, Fenofibrate and Indomethacin after Wet Beads Milling

| Dispersing agents | Nifedipine | Fenofibrate | Indomethacin |
|-------------------|------------|-------------|--------------|
|                   | Z-Average | PDI [-] | Zeta potential [mV] | Z-Average | PDI [-] | Zeta potential [mV] | Z-Average | PDI [-] | Zeta potential [mV] |
| HPC-SSL           | 197       | 0.20     | −32.1         | 186       | 0.25     | −29.7         | 167       | 0.26     | −24.8         |
| HPC-L             | 220       | 0.25     | −30.3         | 213       | 0.28     | −30.3         | 157       | 0.22     | −30.0         |
| HPMC TC-5E        | 231       | 0.24     | −27.0         | 184       | 0.17     | −25.6         | 247       | 0.20     | −23.9         |
| HPMC TC-5R        | 279       | 0.30     | −26.8         | 229       | 0.21     | −46.2         | 264       | 0.21     | −54.7         |
| PVA EG-03         | 212       | 0.10     | −16.6         | 274       | 0.21     | −27.7         | 196       | 0.12     | −28.0         |
| PVA EG-05         | 268       | 0.14     | −23.2         | 282       | 0.25     | −32.4         | 220       | 0.13     | −24.3         |
| PVA EG-18         | 272       | 0.13     | −12.5         | 476       | 0.39     | −21.4         | 345       | 0.24     | −24.5         |
| PVA L-3266        | 240       | 0.23     | −51.9         | 336       | 0.32     | −44.5         | 227       | 0.19     | −44.0         |
| PVP K30           | 189       | 0.14     | −28.1         | 272       | 0.24     | −27.8         | 176       | 0.19     | −27.6         |
| PVP K90           | 289       | 0.24     | −15.6         | 373       | 0.31     | −33.2         | 163       | 0.21     | −38.3         |

*Bold font shows out of CQA (Z-average less than 500 nm and PDI less than 0.3).
HPMC-48 mN/m, PVP-69 mN/m, and PVA-74 mN/m regardless of polymer concentration.²⁹ Given that HPC had low surface tension, it could be derived that it is the appropriate polymer for wet-beads milling. On the other hand, log P, melting point, and enthalpy of fusion are key properties of a drug for decision criteria to obtain nanocrystal.²⁹ Only fenofibrate showed low melting points (> 100°C) and Clog P more than 5. It was suggested that the interaction between the stabilizer and fenofibrate was different compared with nifedipine and indomethacin. However, HPC-SSL was the common dispersing agent identified and all the nanocrystals met CQA. Therefore, HPC-SSL may probably be the universal dispersing agent for nanocrystal suspension preparations.

**Zeta Potential of Freshly Milled Product** Surface charge and electrostatic repulsion are also important to prevent the nanocrystal from forming agglomerates. Zeta potential value is frequently used as an indicator of stability. The effect of polymer on zeta potential is shown in Table 4. It was found that all nanocrystal suspensions were in the anionic state. Most of the measured zeta potential values were −20 mV, and there were no suspensions more than −10 mV. PVA L-3266 showed significantly high value since only PVA L-3266 was anionic polymer while other polymers were non-ionic polymer. It was reported that measured zeta potentials of around −20 mV are considered indicative of a fully stabilized system.²⁹ Moreover, it was common that nanocrystal, colloidal particles or liposome were relatively stabilized by high surface tension, it could be derived that it is the appropriate polymer for wet-beads milling. On the other hand, log P, melting point, and enthalpy of fusion are key properties of a drug for decision criteria to obtain nanocrystal.²⁹

*Polymer Absorption and Stabilization Mechanism* The polymer absorption amount was investigated to deeply clarify the stabilization mechanism by the polymer. The low viscosity grade of each polymer was focused since the better nanocrystal suspension could be achieved. As shown in Table 5, the polymer absorption was observed except for PVP K30. HPC-SSL showed a good affinity with all API and the absorbed amounts was the highest. This finding supported that HPC-SSL was better to stabilize the nanocrystal suspension. Regarding the PVP K30, it was found that the affinity with fenofibrate or indomethacin were not good. However, there was no correlation with the experimental results since indomethacin could prepare good nanocrystal with PVP K30 as shown in Table 4. Therefore, it was suggested that the stabilization mechanism of nanocrystal was not only the repulsion force by polymer absorption but also the other repulsion force worked. Although the repulsion force by polymer absorption was common theory in the pharmaceutical research, no interaction between API and polymer by Fourier transform (FT)-IR analysis,²⁷ raman analysis⁴⁷ or NMR analysis⁴⁸ was also observed in the nanocrystal suspension using similar stabilizer. These finding supported that the other repulsion force also worked as nanocrystal stabilizer.

**Re-dispersing Stability** Soon after each nanocrystal suspensions were diluted into JP1 and JP2, re-dispersion stability was evaluated by the change of Z-average and PDI. These results are shown in Table 6. It was found that re-dispersion stability highly depended on the type of polymer and API. Nifedipine nanocrystal suspensions prepared by HPC-L, HPMC TC-5E, HPMC TC-5R and PVP K90 became agglomerated in JP1 and/or JP2, not meeting CQA. On the other hand, fenofibrate nanocrystal suspensions were stabilized by HPC-L and HPMC TC-TE both in JP1 and JP2. The fenofibrate nanocrystal with HPC-SSL, PVA EG-03, and PVA EG-05 also met the CQA. Indomethacin nanocrystal with HPC-L, HPMC TC-5R and PVP K90 formed aggregates in JP1. Most of the nanocrystal suspensions strongly showed aggregation in JP2. As indomethacin has pH-dependent solubility, solubility under neutral condition (pH 6.8) was approximately 100-fold higher than the one under acid condition (pH 1.2). Indomethacin might be partially dissolved after re-dispersing the nanocrystal suspension into JP2. When the balance of equilibrium

*Bold font shows out of CQA (Z-average less than 500 nm and PDI less than 0.3).*
state collapsed, the amount of precipitated indomethacin was increased. As a result, particle size was greatly increased not only by aggregated nanocrystal but also re-crystalized indomethacin. It implied that there was a potential risk of wide variation of absorption enhancement effect depending on the gastric pH in human. It was thus suggested that the compounds which had pH-dependent solubility in physiological pH condition were very difficult to formulate nanocrystal suspension for bioavailability enhancement. However, it was surprising that the nanocrystal suspensions prepared by HPC-SSL and PVA EG-03 were stable against JP1 and JP2 as compared with other nanocrystal suspensions. On the other hand, the zeta potential of freshly milled products was not related to re-dispersing results. These results indicate that dispersing agent dominates anti-aggregation effect on nanocrystal suspension stability. That means steric hindrance effect by the polymer absorbed on the particle surface mainly contribute to re-dispersion stability than electrostatic effect by the surfactant. Furthermore, they suggest that HPC-SSL is a superior polymer to perform anti-aggregation effect against pH, ionic, and salt concentration stress. Therefore, HPC-SSL not only stabilizes the nanocrystal but also helps obtain smaller and shaper particle size distribution, and thus, it has the potential to be the universal dispersing agent for nanocrystal approaches.

**Wetting Agent Screening**

**Particle Size Distribution of Freshly Milled Product**

Z-Average less than 500 nm and a PDI less than 0.3 were considered as the acceptable CQA for successful nanocrystal and dispersing agent screening. HPC-SSL had the advantages to obtain smaller particle size and showed better stability against JP1/JP2 regardless of polymer type. Therefore, the effect of wetting agents was investigated on Z-average and PDI using three different APIs nifedipine, fenofibrate and indomethacin when HPC-SSL was used (Table 7). As for nifedipine, the nanocrystal suspension observed a Z-average less than 300 nm. Particularly, nanocrystal suspension with DOSS and SLS achieved a Z-average less than 200 nm. However, CO-35 did not meet the acceptable CQA since PDI was more than 0.3. Regarding fenofibrate, the nanocrystal suspension with DOSS, SLS and CTAB met the acceptable CQA. As for indomethacin, all nanocrystal suspensions could achieve Z-average less than 300 nm. However, the nanocrystal with PS80 did not meet the acceptable CQA since PDI was more than 0.3. These results suggested that ionic surfactants were better than nonionic surfactants to achieve a smaller particle size. From the viewpoint of manufacturability, CTAB made foam in nanocrystal suspension during the wet-beads milling process, creating difficulties in preparation. Thus, DOSS and SLS were the effective wetting agents.

**Zeta Potential of Freshly Milled Product**

The effect of wetting agents on zeta potential is shown in Table 7. All nanocrystal suspensions had anionic charge except for fenofibrate nanocrystal suspension prepared by CTAB. Zeta potential mainly correlated with wetting agents rather than API. Moreover, the absolute zeta potential of all nanocrystal suspensions prepared by CTAB was less than 10 mV. Typically, these suspensions were categorized into highly unstable groups, although these suspensions met the CQA. This result suggested that zeta potential was not an important indicator for nanocrystal stability.

**Re-dispersing Stability**

Soon after each nanocrystal suspensions were diluted into JP1 and JP2, the re-dispersion stability was evaluated by the change of Z-average and PDI. These results are shown in Table 8.

In the case of nifedipine and fenofibrate, all nanocrystal suspensions had good re-dispersion stability against JP1 and

---

**Table 7. Z-Average, PDI and Zeta Potential under Various Kinds of Wetting Agents with Nifedipine, Fenofibrate and Indomethacin after Wet Beads Milling**

| Wetting agents | Nifedipine | | | Fenofibrate | | | Indomethacin | | |
|----------------|------------|-----------------|-----------------|----------------|-----------------|----------------|-----------------|-----------------|
|                | Z-Average [nm] | PDI [-] | Zeta potential [mV] | Z-Average [nm] | PDI [-] | Zeta potential [mV] | Z-Average [nm] | PDI [-] | Zeta potential [mV] |
| DOSS           | 197        | 0.20           | −32.1           | 186            | 0.25         | −29.7           | 167            | 0.26         | −24.8           |
| SLS            | 156        | 0.14           | −38.9           | 198            | 0.28         | −38.0           | 140            | 0.20         | −36.7           |
| CTAB           | 272        | 0.24           | −7.9            | 302            | 0.25         | 2.4             | 126            | 0.19         | −10.9           |
| PS80           | 210        | 0.13           | −19.5           | 297            | 0.33         | −15.7           | 224            | 0.34         | −21.9           |
| CO-35          | 276        | **0.31**       | −38.6           | 350            | **0.34**     | −22.8           | 278            | 0.29         | −36.3           |

* Bold font shows out of CQA (Z-average less than 500 nm and PDI less than 0.3).

---

**Table 8. Z-Average and PDI under Various Kinds of Dispersing Agents with Nifedipine, Fenofibrate and Indomethacin against JP1 and JP2**

| Dispersing agents | Nifedipine | | | Fenofibrate | | | Indomethacin | | |
|-------------------|------------|-----------------|-----------------|----------------|-----------------|----------------|-----------------|-----------------|
|                   | Z-Average [nm] | PDI [-] | Z-Average [nm] | PDI [-] | Z-Average [nm] | PDI [-] | Z-Average [nm] | PDI [-] |
| DOSS              | 220        | 0.25           | 215             | 0.26         | 184            | 0.24           | 176             | 0.21           |
| SLS               | 186        | 0.18           | 178             | 0.17         | 174            | 0.20           | 170             | 0.20           |
| CTAB              | 208        | 0.20           | 221             | 0.23         | 233            | 0.21           | 303             | **0.31**       |
| PS80              | 229        | 0.19           | 234             | 0.16         | 167            | 0.21           | 264             | 0.21           |
| CO-35             | 251        | 0.24           | 242             | 0.21         | 361            | 0.27           | 432             | **0.44**       |

* Bold font shows out of CQA (Z-average less than 500 nm and PDI less than 0.3).
JP2 since the aggregation between nanocrystals did not occur even in CTAB with absolute zeta potential less than 10mV. This result strongly supported that zeta potential was not a strong indicator of dispersing stability. On the other hand, indomethacin nanocrystal suspensions with any of the used surfactants aggregated at neutral and/or basic condition due to pH-dependent solubility. However, the impact of aggregation was kept to a minimum when anionic surfactant was used, meeting the CQA. Therefore, DOSS was found to stabilize the nanocrystal in addition to obtaining smaller and shaper particle size distribution, indicating its potential to be used as a universal wetting agent for nanocrystal suspensions.

Physicochemical Property Assessment of Nanocrystal Suspension Using a Selected Stabilizer Combination (HPC-SSL/DOSS)

As for the candidate universal formulation for nanocrystal suspension preparation, the HPC-SSL and DOSS were superior to other combinations, throughout polymer and surfactant screening studies. The SEM pictures of nifedipine, fenofibrate and indomethacin after the wet-beads milling are shown in Fig. 2. These pictures supported that the nanocrystal APIs were successfully obtained using the selected stabilizer combination.

The PXRD pattern of nifedipine, fenofibrate and indomethacin before and after the wet-beads milling are shown in Fig. 3. These results showed that all APIs maintained the original crystalline state even after the wet-beads milling as a hallow pattern was not observed. It is well known that friction heat generated by the hard collision between zirconium balls during the wet-beads milling process increases the product temperature, it was difficult to treat the compound with low melting temperature. However, to overcome this problem, we added a freezing step between the milling steps. PXRD pat-
tern and peak position were not changed by the wet beads milling; therefore, the crystal transitions were not observed. The decrease in peak height and intensity suggested that the crystallinity dropped slightly by the wet beads milling process although crystalline states were kept constant.

The DSC pattern of nifedipine, fenofibrate and indomethacin before and after the wet-beads milling are shown in Fig. 4. All APIs maintained the crystal state even after the wet-beads milling since the melting point of APIs was observed. However, the melting point of APIs got lower after wet-beads milling and peak shape became a broad. These results showed that a part of the APIs crystal interacted with a polymer to make an amorphous state.

**Stability Study**  Stability studies were performed according to ICH Q1A guideline. As shown in Table 9, it was found that nifedipine nanocrystal was stable for 6 months at 40°C. Stability studies indicated that the combination of HPC-SSL and DOSS are applicable for pharmaceutical use.

**Criteria of API Physicochemical Property for Nanocrystal in Oral Delivery**  When nanocrystal suspension is applied to compound development for oral delivery, the criteria from API physicochemical properties are required to assess the feasibility of nanocrystals. The API is much precious at preclinical or early development stage as it reduces the development cost and accelerates development schedule.

The nanocrystal suspensions of each seven water-insoluble compounds were prepared by the wet-beads milling using our proposed stabilizer of 4% (w/v) HPC-SSL and 0.2% (w/v) DOSS. The API physicochemical properties were summarized in Table 10. Z-average and PDI of freshly milled products (water) and re-dispersion stability against JP1 and JP2 are shown in Table 11. Here, the milling experiment results used

### Table 9. The Accelerated Stability Study Results of Nifedipine Nanocrystal Suspension Formulated by HPC-SSL and DOSS

| Compound          | Water         | Z-Average [nm] | PDI [-] | Zeta potential [mV] | Z-Average [nm] | PDI [-] | Z-Average [nm] | PDI [-] |
|-------------------|---------------|----------------|---------|---------------------|----------------|---------|----------------|---------|
|                   | Initial 1M 3M 6M | 1M 3M 6M 1M 3M |         |                     | 1M 3M 6M 1M 3M |         | 1M 3M 6M 1M 3M |         |
| Nifedipine        | 183 191 216   | 0.21 0.19 0.20 | -33.9   | 196 202 218         | 0.21 0.20 0.30 | 190     | 0.20           |
| Phenytin          | 252 259      | 0.29 0.30     | 180 170 | 4240                | 289 280       | 0.23 0.23| 176 52          |
| Fenofibrate       | 360 78       | 0.08 0.3      | 27.3    | 28.5 2.47           | 3.0 3.8         | 4.18    | 3.5            |
| Cilostazol        | 369.5 158–162| 3.0 4.29      | 38.3 8.4| 3.36 3.53           | 3.0 3.9        | 4.18    | 3.4            |
| Bezafibrate       | 361.8 182–186| 34.3 2.54     | 5.7 0.05| 4.37 2.78           | 3.0 3.9        | 4.18    | 3.4            |
| Indomethacin      | 357.8 159    | 2.5 0.7       | 762     | 4.18 3.53           | 3.0 3.9        | 4.18    | 3.4            |
| Itraconazole      | 705.7 168    | 4.5 0.3      | 3.27    | 5.99 0.55           | 3.0 3.9        | 4.18    | 3.4            |
| Piroxicam         | 331.4 200    | 7.3 200       | 660     | 1.29 0.11           | 3.0 3.9        | 4.18    | 3.4            |
| Dipyridamole      | 504.6 165–169| 7.0 100000    | 5.0 3.71| 1.49 2.49           | 3.0 3.9        | 4.18    | 3.4            |
| Ketoprofen        | 254.3 94–97  | 180 170       | 4240    | -0.01 2.76          | 3.0 3.9        | 4.18    | 3.4            |

### Table 10. Physicochemical Properties of Drug Substances

| Compound          | MW [g/mol] | MP [°C] | Solubility [µg/mL] | Log Ds | Log P | Log Ds/P | HPA | HBD | PSA | PSAD |
|-------------------|------------|---------|-------------------|--------|-------|----------|-----|-----|-----|------|
| Nifedipine        | 346.3      | 171     | 6.0               | 7.7    | 7.3   | 2.80     | 3.13| 0.89| 5   | 1    |
| Phenytin          | 252.3      | 295     | 29.3              | 27.3   | 28.5  | 2.47     | 2.09| 1.18| 2   | 2    |
| Fenofibrate       | 360.8      | 78      | 0.8               | 0.3    | 0.3   | 4.80     | 5.23| 0.92| 3   | 0    |
| Cilostazol        | 369.5      | 158–162 | 3.0               | 4.29   | 3.88  | 3.36     | 3.53| 0.95| 5   | 1    |
| Bezafibrate       | 361.8      | 182–186 | 34.3              | 2.54   | 2.05  | -0.17    | 3.70| 0.05| 4   | 2    |
| Indomethacin      | 357.8      | 159     | 2.5               | 0.7    | 762   | 0.77     | 4.18| 0.18| 3   | 1    |
| Itraconazole      | 705.7      | 168     | 0.001             | 4.5    | 0.3   | 3.27     | 5.99| 0.55| 9   | 0    |
| Piroxicam         | 331.4      | 200     | 7.3               | 200    | 660   | 0.20     | 1.29| 0.11| 5   | 2    |
| Dipyridamole      | 504.6      | 165–169 | 7.0               | 100000 | 5.0   | 3.71     | 1.49| 2.49| 12  | 4    |
| Ketoprofen        | 254.3      | 94–97   | 180               | 170    | 4240  | -0.01    | 2.76| 0.00| 3   | 1    |

(a) Refer to L.Z. Benet et al., *The AAPS Journal*, Vol. 13, No. 4, December 2011. (b) Refer to pubchem. https://pubchem.ncbi.nlm.nih.gov/. (c) Refer to interview form. (d) Calculated by MW:PSA. (e) Refer to Table 1. (f) × 100.

### Table 11. Z-Average, PDI and Zeta Potential under Various Kinds of API Formulated by HPC-SSL and DOSS of Freshly Milled Products (Water) and Re-dispersion Stability against JP1 and JP2

| Compound          | Water | Z-Average [nm] | PDI [-] | Zeta potential [mV] | Water | Z-Average [nm] | PDI [-] | Zeta potential [mV] |
|-------------------|-------|----------------|---------|---------------------|-------|----------------|---------|---------------------|
| Nifedipine        | 197   | 0.20           | -32.1   | 220                 | 0.25  | 215            | 0.26    |
| Phenytin          | 170   | 0.17           | -34.2   | 181                 | 0.14  | 183            | 0.19    |
| Fenofibrate       | 186   | 0.25           | -29.7   | 184                 | 0.24  | 176            | 0.21    |
| Cilostazol        | 176   | 0.17           | -31.5   | 375                 | 0.21  | 240            | 0.20    |
| Bezafibrate       | 252   | 0.18           | -26.0   | 274                 | 0.27  | 52             | 0.77    |
| Indomethacin      | 167   | 0.26           | -24.8   | 178                 | 0.24  | 237            | 0.25    |
| Itraconazole      | 186   | 0.20           | -44.7   | 955                 | 0.24  | 506            | 0.24    |
| Piroxicam         | 186   | 0.28           | -24.1   | 1385                | 0.50  | N/A            | N/A     |
| Dipyridamole      | 215   | 0.22           | -29.4   | 3313                | 0.16  | 1096           | 0.26    |
| Ketoprofen        | 349   | 0.68           | -4.7    | 345                 | 0.45  | 334            | 0.71    |

* Bold font shows out of CQA (Z-average less than 500nm and PDI less than 0.3).
a representative data since the reproducibility of the obtained results were very good as shown in Table 12. As for the freshly milled products, most of the nanocrystal suspensions achieved the acceptable CQA except for ketoprofen. As shown in Table 11, all nanocrystal suspensions had anionic charge. Most of the measured zeta potential values were larger than −20 mV; however, only ketoprofen showed the value smaller than −10 mV, indicating lesser stability. However, as we discussed in the previous section, zeta potential might not be the critical factor to obtain stable nanocrystal suspension. That is, zeta potential is not necessary as the criteria of nanocrystal suspension preparation. Looking at API physicochemical properties shown in Table 10, it can be understood that the solubility in water was the only critical factor although there was no correlation among $M_w$, $M_p$, $C \log P$, $D_{2.4}$, hydrogen bonding acceptor (HBA), hydrogen bonding donor (HBD), polar surface area (PSA), and polar surface area density (PSAD). The solubility in water of ketoprofen was $180 \, \mu g/mL$; however, all others had a solubility value less than $35 \, \mu g/mL$.

As for re-dispersion stability against JP1, nifedipine, phenytoin, fenofibrate, cilostazol, bezafibrate, and indomethacin met the CQA. On the other hand, itraconazole, piroxicam, dipyridamole, and indomethacin did not meet the CQA. Most nanocrystals were stable when the solubility at pH 1.2 was less than 35 µg/mL. However, indomethacin, etoricoxib, and dipyridamole did not comply with this rule so that second criteria was sought. Consequently, the criteria of API physicochemical properties for nanocrystal suspension in oral delivery were as follows: Solubility at pH 1.2 and pH 6.8; less than 35 µg/mL, $M_w$; less than 500, the ratio of $\log D_{2.4}$ to $C \log P$; 1 ± 0.2, and HBA; less than 5, respectively.

### Conclusion

In this study, the solubilized formulation of water-insoluble compounds prepared by the wet-beads milling method was proposed. $Z$-average less than 500 nm and a PDI less than 0.3 were defined as acceptable CQA of nanocrystal formulation. To make a clear of stabilization mechanism of nanocrystal suspension, the correlations between API physicochemical properties and dispersing agents or wetting agents were investigated using APIs of three different $pK_a$. In dispersing agent screening study, freshly milled products and re-dispersion stability results concluded that HPC-SSL was better than other structural polymers such as HPMC, PVP and PVA. As for the wetting agents screening study, nonionic surfactants had a tendency to be easily aggregated. On the other hand, ionic surfactants contributed to the stabilization of the nanocrystal. Given the re-dispersion stability and manufacturability, DOSS was the best surfactant. Through the screening study of dispersing agent and wetting agent, we found that the interaction force between dispersing agents and API was stronger than the surface charge such as zeta potential because there was no correlation between zeta potential and the CQA of freshly milled products.

A universal nanocrystal formulation is proposed with the following composition: 20% (w/v) API, 4% (w/v) HPC-SSL and 0.2% (w/v) DOSS. This formulation could be applied in various cases where API water solubility is less than 35 µg/mL. However, for oral delivery additional requirements are to be considered to prevent aggregation in GI tract. We believe that these findings could help pharmaceutical scientists to make better decisions regarding nanocrystal approaches.

### Conflict of Interest

The authors declare no conflict of interest.

### References

1. Loftsson T., Brewster M. E., *J. Pharm. Pharmacol.*, **62**, 1607–1621 (2010).
2. Cooper E. R., *J. Control. Release*, **141**, 300–302 (2010).
3. Merisko-Liversidge E., Liversidge G. G., *Adv. Drug Deliv. Rev.*, **63**, 427–440 (2011).
4. Vioglio P. C., Chierotti M. R., Gobetto R., *Adv. Drug Deliv. Rev.*, **117**, 86–110 (2017).
5. Stella V. J., Nti-Addae K. W., *Adv. Drug Deliv. Rev.*, **59**, 677–694 (2007).
6. Yecom D. W., Son H. Y., Kim J. H., Kim S. R., Lee S. G., Song S.
