International Journal of Pharmaceutics 399 (2010) 52–59

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

High-throughput cocrystal slurry screening by use of in situ Raman microscopy and multi-well plate

Takashi Kojima*, Shunichirou Tsutsumi, Katsuhiko Yamamoto, Yukihiro Ikeda, Toshiya Moriwaki

Pharmaceutical Research Division, Takeda Pharmaceutical Company Ltd., 2-17-85, Jusohonmachi, Yodogawa-ku, Osaka 532-8686, Japan

ARTICLE INFO

Article history:
Received 28 May 2010
Received in revised form 23 July 2010
Accepted 30 July 2010
Available online 7 August 2010

Keywords:
High-throughput screening
Raman spectroscopy
Cocrystal
Crystallization
Polymorphism
Indomethacin

ABSTRACT

Cocrystal has attracted much attention in order to improve poor physicochemical properties, since cocrystal former crystallize with the ionic drugs as well as nonionic drugs. Cocrystal screening was usually conducted by crystallization, slurry and co-grinding techniques, however sensitivity, cost and time for screening were limited because of issues such as dissociation of cocrystal during crystallization and cost and time required for slurry and co-grinding methods. To overcome these issues, novel high-throughput cocrystal slurry screening was developed by using in situ Raman microscope and a multi-well plate. Cocrystal screening of indomethacin was conducted with 46 cocrystal formers and potential cocrystals were prepared on a large scale for the characterization with powder X-ray diffractometry, thermal analysis, and Raman microscopy and ^{1}H NMR spectroscopy. Compared with the characterization of scale-up cocrystals, the cocrystal screening indicated that indomethacin structured novel cocrystals with D/L-mandelic acid, nicotinamide, lactamide and benzamide which was not obtained in the screening with crystallization technique previously reported. In addition, the screening provided not only information of cocrystal formation within a day but also information of equilibrium of cocrystal formation and polymorphic transformation in one screening. Information obtained in this screening allows effective solid form selection by saving cost and time for the development.

1. Introduction

Solid form selection is recognized as an essential process for the pharmaceutical development, since the appropriate solid form could realize to improve poor physicochemical properties such as solubility, stability and crystallinity (Gould, 1986; Ikeda et al., 2008; Kojima et al., 2008; Morris et al., 1994; Tong and Whitesell, 1998). Solid form selection is generally composed of characterization and screenings such as polymorph, salt and cocrystal screening. Especially, cocrystal screening has attracted much attention, since cocrystal former crystallize with the ionic drugs as well as nonionic drugs (Schultheiss and Newman, 2009; Vishweshwar et al., 2006).

Cocrystal is prepared by the crystallization techniques such as solvent evaporation (Basavoju et al., 2008), cooling and adding anti-solvent, slurry technique (Zhang et al., 2007) and co-grinding technique (Trask et al., 2004). Although slurry and co-grinding techniques were reported as the most promising methods to reveal cocrystal formation (Zhang et al., 2007), high-throughput cocrystal screening by using a multi-well plate was mostly conducted by the crystallization techniques in the same manner as the high-throughput salt screening widely used (Carlson et al., 2003; Morissette et al., 2004; Remenar et al., 2003), since slurry and co-grinding methods were usually conducted in a vial with requiring large amount of bulk. In order to improve the issue of amount of bulk, research on slurry technique with the small-scale vial was reported (Takata et al., 2008).

In the field of high-throughput solid form screenings including cocrystal screening, powder X-ray diffractometry (PXRD) and Raman microscopy were commonly used for the analytical method with a multi-well plate (Carlson et al., 2003; Morissette et al., 2003; Peterson et al., 2002). Especially, Raman microscopy is technique for salt and cocrystal screening, since spectra obtained provided not only physical information of polymorphism but also chemical information of salt and cocrystal formation (Kojima et al., 2006). Raman microscopy was also used for in situ analysis which could evaluate time-dependent transformation of crystals such as polymorphic conversion and cocrystal formation during the reaction in slurry (Anquetil et al., 2003; Wikstrom et al., 2009). Recently, application of Raman microscopy for the characterization of cocrystal formation was reported (Rodriguez-Hornedo et al., 2006). However, in situ Raman microscopy has not been applied for the high-throughput cocrystal screen with a multi-well plate up to now.

This paper focuses on the high-throughput cocrystal screening with a multi-well plate and in situ Raman microscopy

* Corresponding author. Fax: +81 6 6308 9019.
E-mail address: Kojima_Takashi@takeda.co.jp (T. Kojima).

0378-5173/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2010.07.055

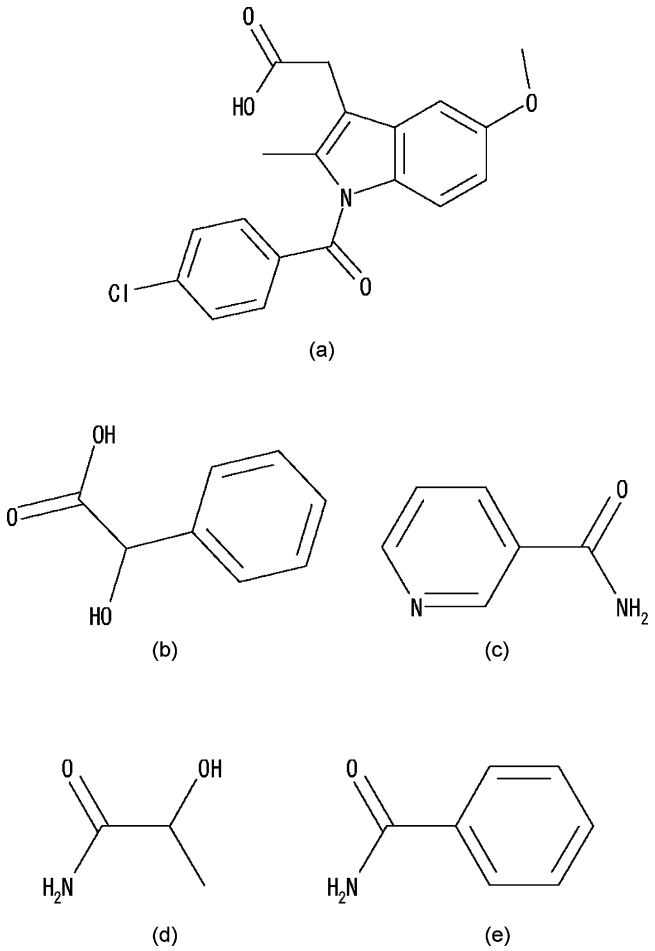

Fig. 1. Chemical structure of indomethacin (a) and cocrystal formers; D/L-mandelic acid (b), nicotinamide (c), lactamide (d) and benzamide (e).

using indomethacin (Fig. 1) as a model drug. Indomethacin is clinically used as a nonsteroidal drug with anti-inflammatory (NSAID), antipyretic and analgesic property and few cocrystal of indomethacin was reported previously (Alleso et al., 2008; Basavoju et al., 2008; Umeda et al., 2009).

In this study, we performed high-throughput cocrystal slurry screening of indomethacin by using in situ Raman microscopy and a multi-well plate. Some novel cocrystals of indomethacin were discovered in a short time through the screening with in situ Raman microscopy and were also separately prepared on a large scale for characterization. In addition, information of cocrystal formation and polymorphic transformation during high-throughput screening was discussed.

2. Materials and methods

2.1. Materials

Indomethacin (form γ) was obtained from Wako Pure Chemical Industries (Osaka, Japan). Indomethacin form α was crystallized from ethanol solution as previously described (Kaneniwa and Otsuka, 1985). Indomethacin 1,4-dioxane solvate was prepared from indomethacin suspended in 1,4-dioxane overnight, filtrated and dried under reduced pressure. Lactamide, naphthalensulfonic acid, S(+) camphor 10-sulfonic acid, ethylmaltol and N-methyl-D-glucamine were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Glycoamide and 1,2-ethandisulfonic acid were obtained from Sigma–Aldrich (St. Louis, MO, USA) and Alfa Aesar (Ward Hill, MA, USA), respectively. The other cocrystal formers were obtained from Wako Pure Chemical Industries (Osaka, Japan). All solvents were purchased from Wako Pure Chemical Industries.

2.2. Cocrystal screening by use of in situ Raman microscopy

Cocrystal screening of indomethacin was conducted using a 96-well plate and Raman microscopy with 46 cocrystal formers such as aromatic acids, carboxylic acids, sulfonic acids, amino acids, amides, amines, sugars and other molecules for potential cocrystal formation. Each 1,4-dioxane solution of indomethacin and cocrystal former was prepared in the concentration of 280 and 126 mM prior to use, respectively. Cocrystal formers which showed low solubility in pure solvent of 1,4-dioxane were dissolved in the mixture of 1,4-dioxane and water to prepare 126 mM solutions on heating. Saturated solutions of cocrystal formers in acetonitrile at the room temperature were also prepared. Each solution of cocrystal former (111 μL) was placed in the column 1, 3, 5, 7, 9 and 11 of the 96-well quartz plate (Hellma, Müllheim, Germany) as listed in Table 1 and solvent was evaporated with nitrogen concentration system, Evan (Moritex, Tokyo, Japan) at room temperature. Solution of indomethacin (50 μL) was also placed in the column 1, 3, 5, 7, 9 and 11 of the 96-well plate to give the binary mixture of indomethacin and cocrystal former in a molar ratio of 1:1. Solvent in the plate was evaporated by nitrogen flow and then evaporated under reduced pressure at the room temperature overnight. Each saturated solution of cocrystal former in acetonitrile (50 μL) was added to the column 1, 3, 5, 7, 9 and 11 of the plate for cocrystal formation and the column 2, 4, 6, 8, 10 and 12 for the reference and indicator to check the precipitate of cocrystal formers during the period of screening. Stir bars (V&P Scientific, San Diego, CA, USA) were placed to all the wells and well plate was sealed with a transparent plate sealer (Greiner Bio-One, Frickenhausen, Germany). The plate was stirred with rotary tumble stirrer VP710C1 (V&P Scientific) at room temperature for 7 days. Saturated solutions of cocrystal former in the column 2, 4, 6, 8, 10 and 12 of the plate were checked for precipitation with a polarized light microscope (PLM). Samples in the column 1, 3, 5, 7, 9 and 11 of the plate were analyzed through the plate sealer with Raman microscope before adding saturated solutions of cocrystal former as initial and 2, 5, 8 h and 1, 2, 3, 4, 7 days after adding solutions.

2.3. Preparation of cocrystals

Indomethacin–D/L-mandelic acid cocrystal (IMC–MDA), indomethacin–nicotinamide cocrystal (IMC–NTA), indomethacin–lactamide cocrystal (IMC–LCA) and indomethacin–benzamide cocrystal (IMC–BZA) were prepared on a 100-mg scale. Indomethacin was suspended in the saturated solution of D/L-mandelic acid, nicotinamide, lactamide or benzamide in acetonitrile. D/L-Mandelic acid, nicotinamide, lactamide or benzamide were added indomethacin suspension of their saturated solution to give 1:1 (indomethacin:cocrystal former) mixtures. The suspension obtained was slurried at room temperature for 6 days, filtrated and dried under reduced pressure. Crystals obtained were subjected to powder X-ray diffractometry (PXRD), thermal analysis, Raman microscopy and ^{1}H NMR analysis.

2.4. Raman microscopy

Raman spectra of slurried samples in the well plate and scaled-up samples were recorded on RXN systems (Kaiser Optical Systems, Ann Arbor MI, USA) at room temperature, equipped with a light-emitting diode laser (785 nm, 400 mW) as an excitation source and an air-cooled CCD detector. A 1-fold objective lens with probe sys-

Table 1
Plate design of cocrystal screening of indomethacin and 46 cocrystal formers.

	1 IMC:CCF (1:1)/sat.	2 Sat. CCF	3 IMC:CCF (1:1)/sat	4 Sat. CCF	5 IMC:CCF (1:1)/sat.	6 Sat. CCF	7 IMC:CF (1:1)/sat.	8 Sat. CCF	9 IMC:CCF (1:1)/sat.	10 Sat. CCF	11 IMC:CCF (1:1)/sat	12 Sat. CCF
A	Hippuric acid		Fumai acid		Maleic acid		p-Toluenesulfonic acid		Nicotinamide		Sucrose	
B	Benzoic acid		L-Tartaric acid		Adipic acid		S(+)Camphor 10-sulfonic acid		Lactamide		Maltose	
C	Gentisic acid		Malonic acid		D/L-Lactic acid		Glycine		Glycolamide		Saccharin	
D	Salicylic acid		Succinic acid		Sorbic acid		L-Tryptophan		Benzamide		Ethylmaltol	
E	D/L-Mandelic acid		Oxalic		Glycolic acid		L-Leucine		Tromethamine		L-Ascorbic acid	
F	1-Hydroxy-2-naphthoic acid		Glutaric acid		Stearic acid		L-Arginine		N-Methyl-D-glucamine		Urea	
G	2,5-Dihydroxybenzoic acid		L-Malic acid		1,2-Ethanedisulfonic add		L-Lysine		D-Mannitol		Reference[a] IMC/MeCN	Reference[b] (MeCN)
H	p-Hydroxybenzoic acid		Citric acid		Naphthalenesulfonic acid		L-Asparatic acid		Lactose		Reference[a] IMC/MeCN	Reference[c] (Stir bar)

Abbreviations used in the table: IMC, indomethacin; CCF, cocrystal former; sat. CCF, saturated solution of CCF; MeCN, acetonitrile.
[a] Slurry of IMC in MeCN for the reference of Raman spectrum.
[b] MeCN for the reference of Raman spectrum.
[c] Stir bar for the reference of Raman spectrum.

Fig. 2. Raman spectra of indomethacin polymorphs and 1,4-dioxane solvate.

tem was used to collect the spectra. The spectra were acquired with 4 cm^{-1} spectral width and 4 s exposure.

2.5. Powder X-ray diffractometry

Powder X-ray diffraction (PXRD) patterns were collected using a RINT 2100 Ultima+ (Rigaku, Tokyo, Japan) with Cu Kα radiation generated at 50 mA and 40 kV. Sample was placed on silicone plate at room temperature. Data was collected from 2 to 35° (2θ) at a step size of 0.02° and scanning speed of 6 °/min.

2.6. Thermal analysis

DSC was performed using a DSC EXTRER 6200 system (Seiko Instruments, Chiba, Japan). A DSC thermogram was obtained in a closed aluminum pan system using a sample weight of ca. 3 mg and a heating rate of 5 °C/min under a nitrogen flow at 50 mL/min. TGA was performed using a TG/DTA EXTER 6200 system (Seiko Instruments). A TGA thermogram was obtained in an open aluminum pan system under the same conditions as those for DSC at 100 mL/min nitrogen flow.

2.7. NMR Spectroscopy

^{1}H NMR data were collected on AV-400 (Bruker Bio Spin, Ettlingen, Germany) operating at 400 MHz. Samples were dissolved in DMSO-*d6*.

3. Results

3.1. Cocrystal screening on 96-well plate by use of in situ Raman microscopy

Slurried mixtures of indomethacin and cocrystal former in the 96-well plate were analyzed by Raman microscopy for 7 days. Raman spectra of binary mixture of indomethacin and cocrystal former in a molar ratio of 1:1 suggested that indomethacin was transformed to new crystalline form without cocrystal formation. PXRD and thermal analysis suggested that new crystalline form obtained in the well plate was hemi-1,4-dioxane solvate (data not shown). In the Raman spectra, stable form γ, form α and 1,4-dioxane solvate showed a sharp band at 1698 cm^{-1}, split band at 1679 and 1689 cm^{-1} and sharp band at 1687 cm^{-1} attributable to the stretching of C=O bond of indomethacin, respectively (Fig. 2).

PLM observations of the saturated cocrystal former solution in the column 2, 4, 6, 8, 10 and 12 suggested that slurried mixture of solid-state indomethacin and cocrystal former in the

Table 2
Wave number of C=O and crystalline form of indomathacin and cocrystal former mixtures slurried for 1 week.

		Initial		1 week slurried	
		Wave number	Crystal form	Wave number	Crystal form
	Indomethacin	1687	Solvate	1698	Form γ
1	Hippuric acid	1687	Solvate	1698	Form γ
2	Benzoic acid	1687	Solvate	1698	Form γ
3	Gentisic acid	1687	Solvate	1698	Form γ
4	Salicylic acid	1687	Solvate	1698	Form γ
5	D/L-Mandelic acid	1687	Solvate	1683	Cocrystal
6	1-Hydroxy-2-naphthoic acid	1687	Solvate	1698	Form γ
7	2,5-Dihydroxybenzoic acid	1687	Solvate	1698	Form γ
8	p-Hydroxybenzoic acid	1687	Solvate	1698	Form γ
9	Fumaric acid	1687	Solvate	1698	Form γ
10	L-Tartaric acid	1687	Solvate	1698	Form γ
11	Malonic acid	1687	Solvate	1698	Form γ
12	Succinic acid	1687	Solvate	1698	Form γ
13	Oxalic acid	1687	Solvate	1698	Form γ
14	Glutaric acid	1687	Solvate	1698	Form γ
15	L-Malic acid	1687	Solvate	1698	Form γ
16	Citric acid	1687	Solvate	1698	Form γ
17	Maleic acid	1687	Solvate	1698	Form γ
18	Adipic acid	1687	Solvate	1698	Form γ
19	D/L-Lactic acid	1687	Solvate	1698	Form γ
20	Sorbic acid	1687	Solvate	1698	Form γ
21	Glycolic acid	1687	Solvate	1698	Form γ
22	Stearic acid	1688	Solvate	1698	Form γ
23	1,2-Ethanedisulfonic acid	1687	Solvate	1698	Form γ
24	Naphthalenesulfonic acid	N.D.	-	1698	Form γ
25	p-Toluenesulfonic acid	N.D.	-	1697	Form γ
26	S(+) Camphor 10-sulfonic acid	1687	Solvate	1697	Form γ
27	Glycine	1687	Solvate	1698	Form γ
28	L-Tryptophan	1687	Solvate	1698	Form γ
29	L-Leucine	1687	Solvate	1698	Form γ
30	L-Arginine	1687	Solvate	1698	Form γ
31	L-Lysine	1687	Solvate	1698	Form γ
32	L-Asparatic acid	1687	Solvate	1698	Form γ
33	Nicotinamide	1687	Solvate	1672	Cocrystal
34	Lactamide	1687	Solvate	1669	Cocrystal
35	Glycolamide	1687	Solvate	1698	Form γ
36	Benzamide	1687	Solvate	1692	Cocrystal
37	Tromethamine	1673	Salt	1676	Salt
38	N-Methyl-D-glucamine	1686	Solvate	1674	Salt
39	D-Mannitol	1687	Solvate	1698	Form γ
40	Lactose	1687	Solvate	1698	Form γ
41	Sucrose	1687	Solvate	1698	Form γ
42	Maltose	1687	Solvate	1698	Form γ
43	Saccharin	1684	Cocrystal	1684	Cocrystal
44	Ethylmaltol	1687	Solvate	1698	Form γ
45	L-Ascorbic acid	1687	Solvate	1698	Form γ
46	Urea	1687	Solvate	1697	Form γ

Solvate: indomethacin 1,4-dioxane solvate, form γ: indomethacin form γ, cocrystal: potential cocrystal of indomethacin and coformer, salt: potential salt of indomethacin and coformer, N.D.: data could not be obtained because of high background.

column 1, 3, 5, 7, 9 and 11 would maintain the molar ratio of 1:1 (indomethacin:cocrystal former) without vaporization of solvent during the screening, since precipitation from saturated solution was not observed in the column 2, 4, 6, 8, 10 and 12 for 7 days.

The results of cocrystal screening of indomethacin on the 96-well plate are shown in Table 2. The Raman spectra of binary mixture of indomethacin and cocrystal former after 7-day slurried were compared with the spectrum of indomethacin form γ, form α and 1,4-dioxane solvate in the shift region 1635–1715 cm^{-1} attributable to the stretching of C=O bond. The Raman spectra indicated that indomethacin could structure cocrystals with D/L-mandelic acid (IMC–MDA), nicotinamide (IMC–NTA), lactamide (IMC–LCA), benzamide (IMC–BZA) and saccharin (IMC–SAC) and form salts with tromethamine and N-methyl-D-glucamine. The spectra of the mixture of indomethacin and eight aromatic acids were shown as an example in Fig. 3. The spectra suggested that only the mixture with D/L-mandelic acid showed the peak at 1683 cm^{-1} which could be assigned as IMC–MDA, and other seven mixtures showed the peaks at 1698 cm^{-1} which were assigned as indomethacin form γ. Indomethacin 1,4-dioxane solvate transformed to stable form γ in the most of mixture of indomethacin and cocrystal former. Only in D/L-lactic acid, polymorphic transformation of indomethacin 1,4-dioxane solvate to stable form γ via metastable form α was observed (Fig. 4).

The Raman spectra of the mixture of indomethacin cocrystal formers, nicotinamide, lactamide and saccharin for 7 days were sorted separately to evaluate the time-dependent transformation (Fig. 5). In the cocrystal screening of indomethacin and nicotinamide, indomethacin 1,4-dioxane solvate and form α mixture were transformed to potential cocrystal of IMC–NTA after 2 h slurring in the nicotinamide saturated acetonitrile solution. In the screening of indomethacin and lactamide, indomethacin 1,4-dioxane solvate was transformed to indomethacin form γ after 2 h slurring and then potential formation of IMC–LCA was observed after 8 h. In contrast, IMC–SAC cocrystal was partially obtained just after evaporation of indomethacin and saccharin solutions

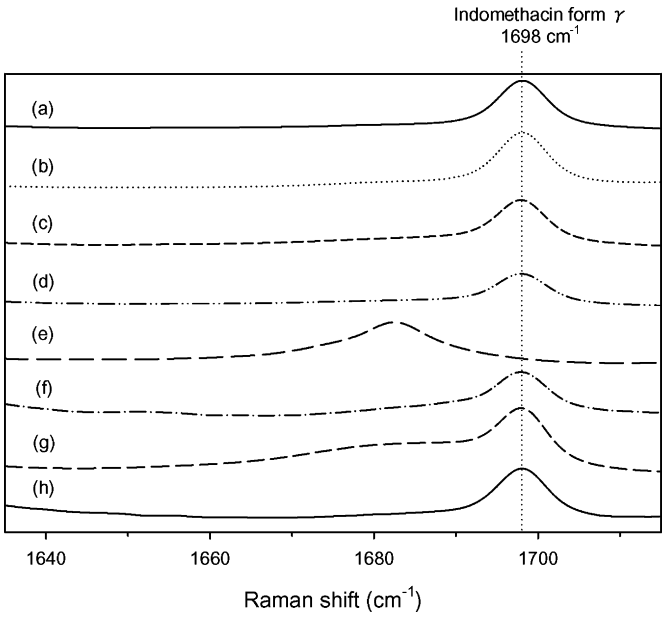

Fig. 3. Raman spectra of indomethacin and aromatic acids slurried in the well plate after 7 days. Aromatic acids were hippuric acid (a); benzoic acid (b); gentisic acid (c); salicylic acid (d); D/L-mandelic acid (e); 1-hydroxy-2-naphthoic acid (f); 2,5-dihydroxybenzoic acid (g); and p-hydroxybenzoic acid (h).

before slurring in the screening of indomethacin and saccharin. Raman spectra suggested that the surface of solid just after evaporation was IMC–SAC. And then, equilibrium of IMC–SAC formation for 1 day was observed, since the mixture of IMC–SAC and 1,4-dioxane solvate were detected by Raman microscopy in 2 and 8 h. The other potential two cocrystals, IMC–MDA and IMC–BZA were transformed after 2 h slurring as same as IMC–NTA cocrystal.

3.2. Characterization of cocrystal by scaled-up preparation

Indomethacin cocrystals obtained in the screening, IMC–MDA, IMC–NTA, IMC–LCA and IMC–BZA were prepared on a 100-mg scale and characterized using PXRD, DSC, TGA, Raman microscope

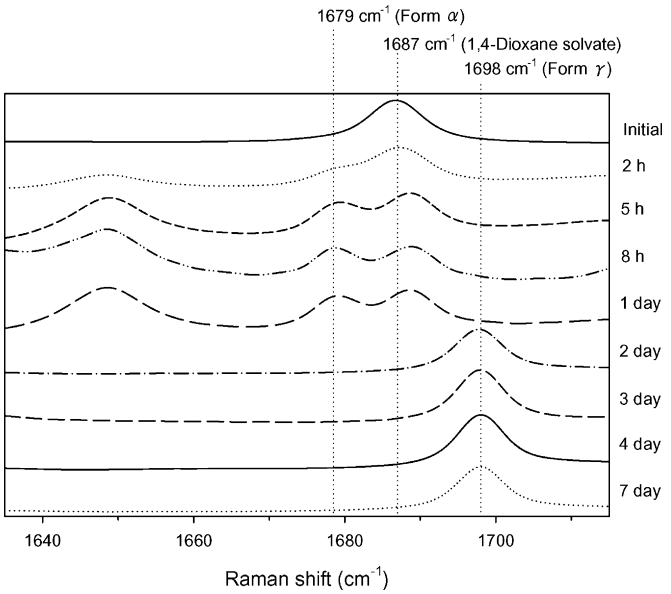

Fig. 4. Raman spectra of indomethacin and D/L-lactic acid in the well plate for 7 days.

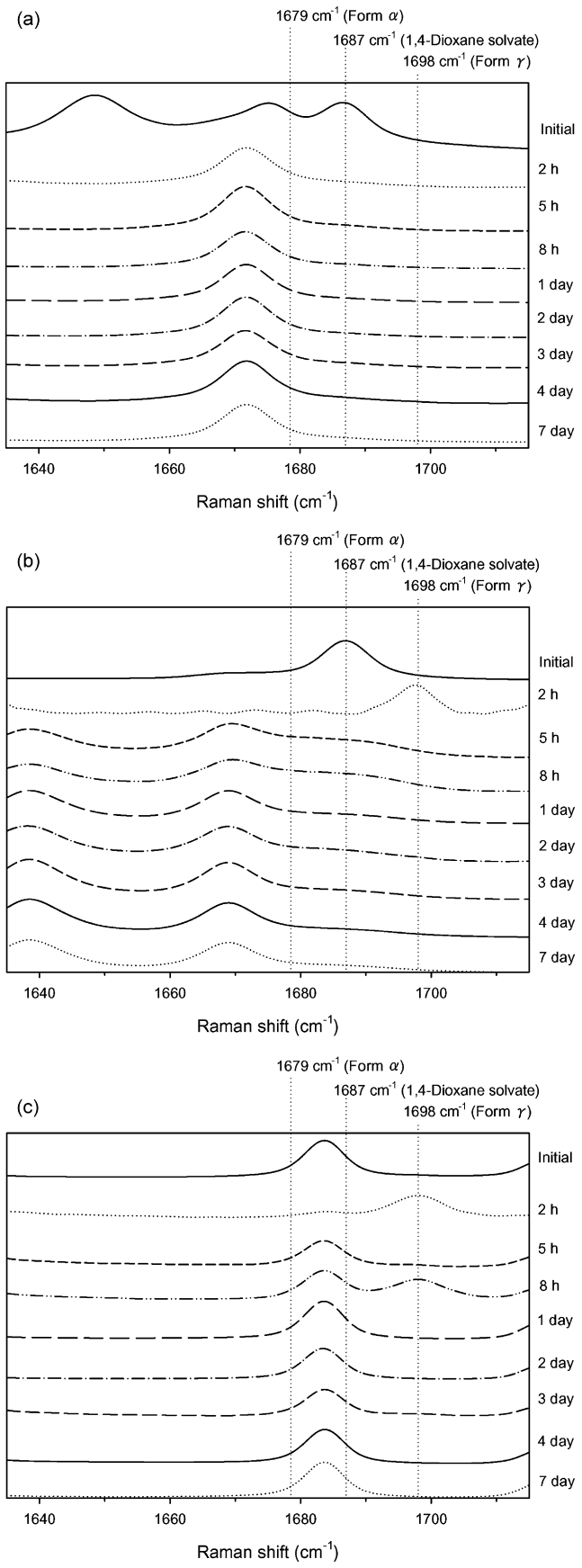

Fig. 5. Raman spectra of indomethacin and nicotinamide (a); lactamide (b); saccharin, (c) in the well plate for 7 days.

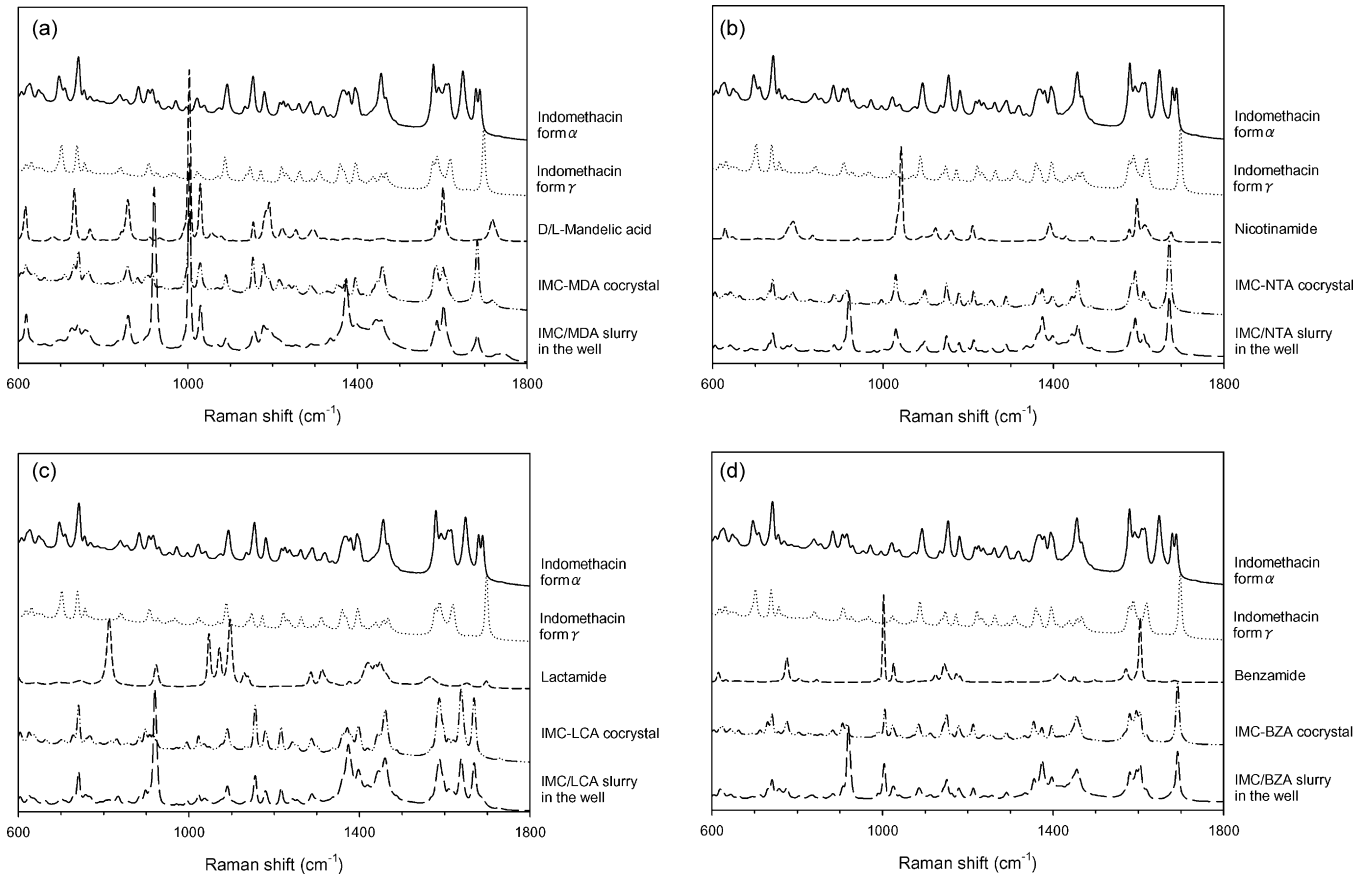

Fig. 6. Raman spectra of scaled-up indomethacin cocrystals and slurried in the well plate for 7 days.

Table 3
Molecular ratio of the components (indomethacin:cocrystal former) and melting points of indomethacin, cocrystal formers and cocrystals.

	Mol. ratio	Melting point (°C)		
		Indomethacin	Coformer	Cocrystal
Indomethacin (forms α and γ)	1:0	153, 159	–	–
Indomethacin–D/L-mandelic acid cocrystal	1:2.5	–	119	106
Indomethacin–nicotinamide cocrystal	1:1	–	128	123
Indomethacin–lactamide cocrystal	1:0.5	–	77	126
Indomethacin–benzamide cocrystal	1:1	–	124	109

and ^{1}H NMR. Raman spectra suggested that prepared sample on a 100-mg scale were different from indomethacin polymorphs and cocrystal formers and would be the same solid forms as 7-day slurried in the 96-well plate (Figs. 6-1–6-4). PXRD patterns also indicated that scaled-up samples were crystalline and different from indomethacin forms α and γ (Fig. 7). Thermal analysis suggested that all the samples prepared were anhydrous form and their melting points were different from indomethacin polymorphs and cocrystal formers (Table 3). The results of ^{1}H NMR indicated that the molecular ratios of cocrystal formers to indomethacin were 2.46, 0.97, 0.50 and 1.02 for IMC–MDA, IMC–NTA, IMC–LCA and IMD–BZA, respectively. Taken together, these results suggested that crystals obtained were indomethacin cocrystals with the stoichiometry of 1:2.5 (IMC:MDA), 1:1 (IMC:NTA), 1:0.5 (IMC:LCA) and 1:1 (IMC:BZA).

Characterization of the cocrystals produced in the scaled-up preparation indicated that potential cocrystals detected in the screening were the same crystalline forms as those of cocrystals prepared in a large scale. These results suggested that cocrystal formation could be evaluated in the 96-well plate as same as scale-up slurry in a vial.

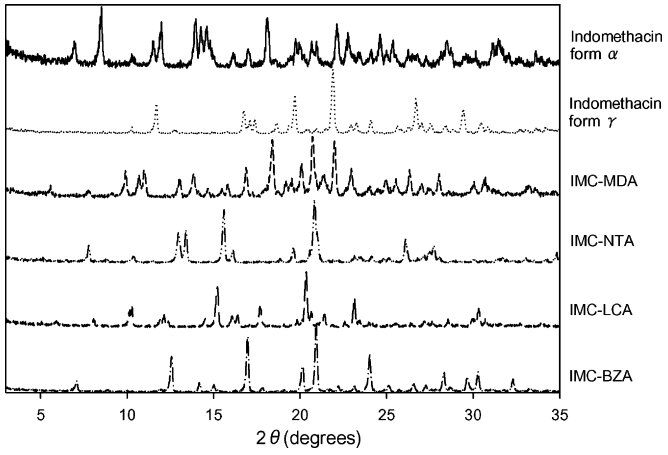

Fig. 7. PXRD patterns of scaled-up indomethacin, cocrystal former, and indomethacin cocrystals.

4. Discussion

We have demonstrated for the first time that high-throughput cocrystal slurry screening using in situ Raman microscope and a multi-well plate can predict the cocrystal formation with high accuracy with a small amount of bulk in a short time. Through the use of techniques we have developed, cocrystal slurry screening with 46 cocrystal formers could be performed with 230 mg of drug candidate within a day and information of time-dependent of cocrystal formation and polymorphic transformation was easily obtained.

To conduct the screening of solid form, sample was generally prepared in the multi-well plate and analyzed by the high-throughput instruments and classification software of data (Barr et al., 2004, 2009), since the numerous combinations of drug candidates, counter molecules and solvents should be evaluated. Especially cocrystal screening should require lots of combination of drug with cocrystal formers including pharmaceutically acceptable excipient. Cocrystal screening is usually conducted by the crystallization techniques such as solvent evaporation, cooling and adding anti-solvent, slurry technique and co-grinding technique. Crystallization techniques were appropriate methods for the high-throughput screening, since they could be conducted in the same manner as the high-throughput salt screening widely used in the pharmaceutical industries. However some cocrystals could not be structured during crystallization and crystallization resulted in the physical mixture of drug and cocrystal former because of their weak interaction between drug and cocrystal former compared to salt formation (Childs et al., 2007). To overcome the issue of dissociation of cocrystal during crystallization, slurry and co-grinding techniques called a lot of attention (Zhang et al., 2007). Even though slurry technique could be applied to the high-throughput cocrystal screening compared to co-grinding technique from the viewpoint of sample preparation and research on small-scale slurry was reported (Takata et al., 2008), slurry screening was still conducted not in a multi-well plate, but in a vial and taking several days to obtain the results.

Powder X-ray diffractometry and Raman microscopy were commonly used as high-throughput instruments for cocrystal screening. Especially, Raman microscopy is useful analysis for cocrystal screening, since spectra obtained provided not only physical information of polymorphism but also chemical information of cocrystal formation (Kojima et al., 2006). In late years, Raman microscopy has been used for in situ analysis of time-dependent crystallization, polymorphic conversion and cocrystal formation (Anquetil et al., 2003; Rodriguez-Hornedo et al., 2006; Wikstrom et al., 2009). The possibility of application of in situ Raman spectroscopy to detect cocrystal formation during slurry cocrystal screening has been also discussed (Zhang et al., 2007). However, to the best of our knowledge, demonstration of the cocrystal screening with in situ Raman microscopy and a multi-well plate has not been conducted. In this study, we performed the high-throughput cocrystal slurry screening on indomethacin by using in situ Raman microscopy and a multi-well plate and not only information of cocrystal formation within a day but also information of equilibrium of cocrystal formation and polymorphic transformation was obtained in one screening. Cocrystal screening of indomethacin has been previously reported by using the technique of crystallization from solution (Basavoju et al., 2008). Even though the screening reported has conducted including nicotinamide and saccharin as cocrystal former, IMC–NTA cocrystal was not obtained and only IMC–SAC cocrystal was crystallized in the their report. Cocrystal slurry screening by using in situ Raman microscopy, we have developed, suggested that IMC–MDA, IMC–NTA, IMC–LCA and IMC–BZA cocrystals were obtained during slurry for 2–8 h, whereas IMC–SAC cocrystal formation was observed just after evaporation of indomethacin and saccharin solutions before slurring. These results suggested that interaction between indomethacin and saccharin was stronger than those of other cocrystal formers in the solution and resulted that the crystallization of IMC–SAC cocrystal could occur by the technique of crystallization from solution. The result also indicated that cocrystal screening by the crystallization technique from solution revealed only one cocrystal, IMC–SAC cocrystal, whereas cocrystal slurry screening by using in situ Raman microscopy revealed IMC–NTA cocrystal which could not be obtained by the crystallization techniques from solution and moreover discovered three more novel cocrystals, IMC–MDA, IMC–LCA and IMC–BZA.

Recently, cocrystal of the same component with different stoichiometry was reported (Trask et al., 2005). In the report, caffeine–maleic acid cocrystal was obtained as two different stoichiometry, 1:1 and 2:1 (caffeine:maleic acid). Cocrystal also has polymorphs and pseudopolymorphs as same as crystals of single component and salt crystals. In the pharmaceutical development, appropriate solid form should be selected depending on the purpose such as improvement of solubility, stability and crystallinity. From the viewpoint of manufacturability and stability, the most thermodynamically stable form was generally selected (Miller et al., 2005). In the case that drug candidate had different stoichiometric cocrystal or polymorphism, the most thermodynamically stable form should be selected appropriately for the development. In this study, polymorphic transformation of indomethacin to the most stable form γ from 1,4-dioxane solvate via metastable form α based on Ostwald's step rule was clearly observed in D/L-lactic acid. This result suggested that cocrystal slurry screening by using in situ Raman microscopy could detect polymorphism and transformation of cocrystal and evaluate the most thermodynamically stable form which would be appropriate for the pharmaceutical development.

In conclusion, our investigation enabled successful development of cocrystal slurry screening by using in situ Raman microscope and a multi-well plate, providing not only information of cocrystal formation within a day but also information of equilibrium of cocrystal formation and polymorphic transformation in one screening. Information obtained in this screening allows effective solid form selection by saving cost and time for the development.

Acknowledgment

We wish to thank Hideko Fukumyo, Discovery Research Center, Takeda Pharmaceutical Company Ltd. for her assistance of ^{1}H NMR analysis.

References

Alleso, M., Velaga, S., Alhalaweh, A., Cornett, C., Rasmussen, M.A., van den Berg, F., de Diego, H.L., Rantanen, J., 2008. Near-infrared spectroscopy for cocrystal screening. A comparative study with Raman spectroscopy. Anal. Chem. 80, 7755–7764.

Anquetil, P.A., Brenan, C.J., Marcolli, C., Hunter, I.W., 2003. Laser Raman spectroscopic analysis of polymorphic forms in microliter fluid volumes. J. Pharm. Sci. 92, 149–160.

Barr, G., Dong, W., Gilmore, C.J., 2004. PolySNAP: a computer program for analyzing high-throughput powder diffraction data. J. Appl. Cryst. 37, 658–664.

Barr, G., Cunningham, G., Dong, W., Gilmore, C., Kojima, T., 2009. High-throughput powder diffraction V: the use of Raman spectroscopy with and without X-ray powder diffraction data. J. Appl. Cryst. 42, 706–714.

Basavoju, S., Bostrom, D., Velaga, S.P., 2008. Indomethacin–saccharin cocrystal: design, synthesis and preliminary pharmaceutical characterization. Pharm. Res. 25, 530–541.

Carlson, E.D., Chandler, W.J., Chau, H.K., Cong, P., Crevier, T., Desrosiers, P.J., 2003. An integrated high throughput workflow for pre-formulations: polymorph and salt selection studies. Pharm. Chem., 10–15.

Childs, S.L., Stahly, G.P., Park, A., 2007. The salt-cocrystal continuum: the influence of crystal structure on ionization state. Mol. Pharm. 4, 323–338.

Gould, P.L., 1986. Salt selection for basic drugs. Int. J. Pharm. 33, 201–217.

Ikeda, Y., Ban, J., Ishikawa, T., Hashiguchi, S., Urayama, S., Horibe, H., 2008. Stability and stabilization studies of TAK-599 (Ceftaroline Fosamil), a novel N-phosphono

type prodrug of anti-methicillin resistant *Staphylococcus aureus* cephalosporin T-91825. Chem. Pharm. Bull. (Tokyo) 56, 1406–1411.
Kaneniwa, N., Otsuka, M., 1985. Physicochemical characterization of indomethacin polymorphs and the transformation kinetics in ethanol. Chem. Pharm. Bull. 33, 3447–3455.
Kojima, T., Onoue, S., Murase, N., Katoh, F., Mano, T., Matsuda, Y., 2006. Crystalline form information from multi-well plate salt screening by use of Raman microscopy. Pharm. Res. 23, 806–812.
Kojima, T., Sugano, K., Onoue, S., Murase, N., Sato, M., Kawabata, Y., Mano, T., 2008. Solid form selection of zwitterionic 5-HT4 receptor agonist. Int. J. Pharm. 350, 35–42.
Miller, J.M., Collman, B.M., Greene, L.R., Grant, D.J., Blackburn, A.C., 2005. Identifying the stable polymorph early in the drug discovery–development process. Pharm. Dev. Technol. 10, 291–297.
Morissette, S.L., Soukasene, S., Levinson, D., Cima, M.J., Almarsson, O., 2003. Elucidation of crystal form diversity of the HIV protease inhibitor ritonavir by high-throughput crystallization. Proc. Natl. Acad. Sci. U.S.A. 100, 2180–2184.
Morissette, S.L., Almarsson, O., Peterson, M.L., Remenar, J.F., Read, M.J., Lemmo, A.V., Ellis, S., Cima, M.J., Gardner, C.R., 2004. High-throughput crystallization: polymorphs, salts, co-crystals and solvates of pharmaceutical solids. Adv. Drug Deliv. Rev. 56, 275–300.
Morris, K.R., Fakes, M.G., Thakur, A.B., Newman, A.W., Singh, A.K., 1994. An integrated approach to the selection of optimal salt form for a new drug candidate. Int. J. Pharm. 105, 209–217.
Peterson, M.L., Morissette, S.L., McNulty, C., Goldsweig, A., Shaw, P., LeQuesne, M., Monagle, J., Encina, N., Marchionna, J., Johnson, A., Gonzalez-Zugasti, J., Lemmo, A.V., Ellis, S.J., Cima, M.J., Almarsson, O., 2002. Iterative high-throughput polymorphism studies on acetaminophen and an experimentally derived structure for form III. J. Am. Chem. Soc. 124, 10958–10959.
Remenar, J.F., MacPhee, J.M., Larson, B.K., Tyagi, V.A., Ho, J.H., 2003. Salt selection and simultaneous polymorphism assessment via high-throughput crystallization: the case of sertraline. Org. Process. Res. Dev. 7, 990–996.
Rodriguez-Hornedo, N., Nehm, S.J., Seefeldt, K.F., Pagan-Torres, Y., Falkiewicz, C.J., 2006. Reaction crystallization of pharmaceutical molecular complexes. Mol. Pharm. 3, 362–367.
Schultheiss, N., Newman, A., 2009. Pharmaceutical cocrystals and their physicochemical properties. Cryst. Growth Des. 9, 2950–2967.
Takata, N., Shiraki, K., Takano, R., Hayashi, Y., Terada, K., 2008. Cocrystal screening of stanolone and mestanolone using slurry crytallization. Cryst. Growth Des. 8, 3032–3037.
Tong, W.Q., Whitesell, G., 1998. In situ salt screening—a useful technique for discovery support and preformulation studies. Pharm. Dev. Technol. 3, 215–223.
Trask, A.V., Motherwell, W.D., Jones, W., 2004. Solvent-drop grinding: green polymorph control of cocrystallisation. Chem. Commun. (Camb.), 890–891.
Trask, A.V., Motherwell, W.D., Jones, W., 2005. Pharmaceutical cocrystallization: engineering a remedy for caffeine hydration. Cryst. Growth Des. 5, 1013–1021.
Umeda, Y., Fukami, T., Furuishi, T., Suzuki, T., Tanjoh, K., Tomono, K., 2009. Characterization of multicomponent crystal formed between indomethacin and lidocaine. Drug Dev. Ind. Pharm. 35, 843–851.
Vishweshwar, P., McMahon, J.A., Bis, J.A., Zaworotko, M.J., 2006. Pharmaceutical cocrystals. J. Pharm. Sci. 95, 499–516.
Wikstrom, H., Kakidas, C., Taylor, L.S., 2009. Determination of hydrate transition temperature using transformation kinetics obtained by Raman spectroscopy. J. Pharm. Biomed. Anal. 49, 247–252.
Zhang, G.G., Henry, R.F., Borchardt, T.B., Lou, X., 2007. Efficient co-crystal screening using solution-mediated phase transformation. J. Pharm. Sci. 96, 990–995.