# Cyclodextrin-based isolation of Ostwald's metastable polymorphs occurring during crystallization

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**We describe a novel approach for the selective isolation of Ostwald's intermediate metastable polymorphs occurring during an early stage of crystallization, by utilizing the inclusion complex formed with a cyclic oligosaccharide derivative, 2,6-di-*O*-methyl-β-cyclodextrin.**

Different crystalline polymorphs exhibit different physicochemical properties such as solubility, dissolution rate, bioavailability, and chemical and physical stabilities. Therefore, it is of great importance to discover, produce and isolate crystalline polymorphs of a given solid drug and to control their polymorphic transitions[1] that proceed generally *via* a solid–solid or a solution-mediated mechanism.[2] The solid–solid transformation is dependent on internal rearrangements or conformational changes of the molecules in crystals. On the other hand, the solution-mediated transformation is controlled by differences in solubility of the stable and metastable forms, where a metastable form with a higher solubility appears first from solution and it then dissolves, nucleates and transforms into a stable form with a lower solubility, according to "Ostwald's Rule of Stages".[3]

Cyclodextrins (CDs), cyclic oligosaccharides consisting of usually 6 to 8 D-glucose units, form inclusion complexes with various molecules in aqueous solution and in the solid state.[4] We expected *a priori* that the solution-mediated polymorphic transformation of compounds will be suppressed by inclusion complex formation with CDs, because CDs generally enhance the solubility of drugs in water, slow down the diffusion rate of drugs due to the increase in molecular volume, and inhibit aggregation of drugs due to the masking of intermolecular interaction sites by inclusion complex formation. On the basis of these premises, we studied effects of CDs on crystallization and polymorphic transformation of the oral hypoglycemic agents tolbutamide, chlorpropamide and acetohexamide in aqueous solution, and report here for the first time that 2,6-di-*O*-methyl-β-CD (DM-β-CD) markedly suppresses the solution-mediated polymorphic transformation of Ostwald's metastable forms to stable forms by inclusion complex formation, yielding exclusively metastable forms. Among the three hypoglycemic drugs, tolbutamide (Fig. 1) was mainly investigated, because its polymorphic forms have been well characterized by us and others.[5]

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The crystallization of the hypoglycemic drugs in the absence and presence of various CDs in aqueous solution was conducted as follows: tolbutamide was dissolved at 5.0 mM concentration in the absence and presence of CDs at various concentrations in pH 8.0 sodium phosphate buffer (20 mL, prepared with 0.1 M $H_3PO_4$–0.1 M NaOH) in a 50 mL beaker at room temperature. The solution was slowly titrated with aqueous 0.5 M HCl solution (about 2 mL) to pH 6.8 where tolbutamide did not yet precipitate. The solution was paper-filtered, and the filtrate was put in a refrigerator (4 °C) for 1 day. The precipitated tolbutamide crystals were collected by filtration, and the contents of different polymorphs were determined by powder X-ray diffraction. The concentrations of tolbutamide in the filtrates were determined by UV spectroscopy at 230 nm.

Fig. 1 shows powder X-ray diffraction patterns of crystals precipitated from aqueous, buffered (pH 6.8) 5 mM tolbutamide solution in the absence and presence of various CDs (5 mM). It is apparent that in the absence of CDs, tolbutamide crystallized into stable Form I, giving the diffraction peaks typical for tolbutamide

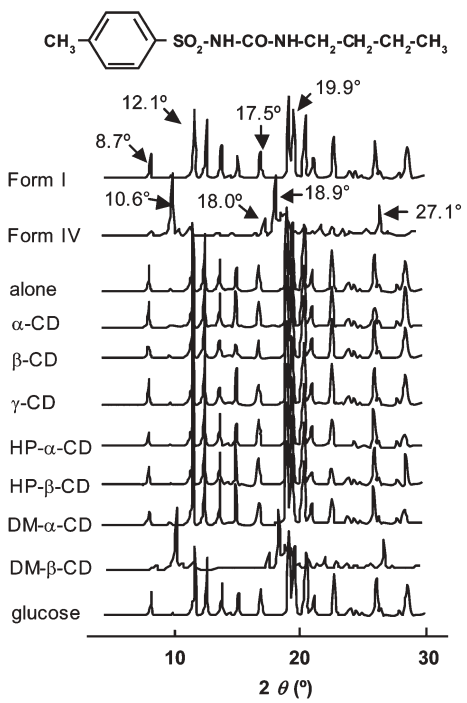



**Fig. 1** Powder X-ray diffraction diagrams of crystals obtained from 5 mM tolbutamide in pH 6.8 sodium phosphate buffer in the absence and presence of 5 mM CDs or 35 mM glucose at 4 °C.

Form I crystals, *e.g.*, $2\theta$ = 8.7, 12.1, 17.5 and 19.9°. Stable Form I crystals of tolbutamide were also obtained from solutions containing 5 mM parent α-, β- and γ-CDs, 2-hydroxypropyl-α-CD (HP-α-CD), HP-β-CD, DM-α-CD, and 35 mM glucose. By sharp contrast, the solution containing 5 mM DM-β-CD yielded exclusively metastable Form IV crystals, which showed a different X-ray diffraction pattern compared to Form I, *e.g.*, the diffraction peaks typical for tolbutamide Form IV crystals at $2\theta$ = 10.6, 18.0, 18.9 and 27.1°. The crystalline polymorphs differed in habits, Form I being plate-like and Form IV needle-like, as shown in Fig. 2. In all cases, tolbutamide crystals were recovered in 60–70% yield of the initially added amounts, and the CDs had not cocrystallized with tolbutamide at the concentrations used (<5 mM CDs), as confirmed by $^1$H-NMR spectroscopy, thin-layer chromatography and elemental analysis. Similarly, chlorpropamide crystallized exclusively in its metastable Form II in the presence of DM-β-CD, and acetohexamide was found in a new form that is different from the reported Form A and B crystals, as will be reported elsewhere.

Figs. 3a and 3b show changes of polymorph contents in the total crystal mass obtained during storage (1 h to 1 week) at 4 °C. From tolbutamide solutions without CDs, metastable Form IV precipitated at initial stages of crystallization, but transformed completely to Form I within 20 h (Fig. 3a). By contrast, in the presence of DM-β-CD, Form I was exclusively crystallized for more than 1 week (Fig. 3b), and even after 2 weeks the conversion of Form IV to Form I was only about 20%. The selective crystallization of the metastable Form IV was observed only above 0.1 mM DM-β-CD concentration, as shown in Fig 4. We investigated how Form IV crystals transform to Form I crystals, when excess amounts (15 mg) of Form IV crystals were added to the saturating tolbutamide solution (0.4 mM, 20 mL) of Form I crystals. As shown in Fig. 5, the added Form IV crystals dissolved in the absence of CDs, because the solubility of Form IV is larger than that of Form I. Therefore, the tolbutamide concentration increased within about 3 h to about 1.4 mM with saturating solubility of 1.3–1.5 mM. However, shortly afterwards it decreased due to the crystallization of Form I, because the concentration is supersaturated with respect to Form I, yielding a final tolbutamide concentration of 0.4–0.5 mM. On the other hand, when the solution contained 5 mM DM-β-CD, the added Form IV crystals dissolved to a tolbutamide concentration of 1.4 mM that remained, as no transformation of the added Form IV crystals to the stable Form I crystals occurred. When inclusion competitors such as *p*-hydoxybenzoic acid esters were added to the tolbutamide–DM-β-CD solution, the crystallization of the metastable Form IV was inhibited, giving stable Form I crystals. These results indicate clearly that the stable Form I of tolbutamide crystallizes *via* the metastable Form IV according to a solution-mediated transition and DM-β-CD inhibits this transition through complexation with tolbutamide.

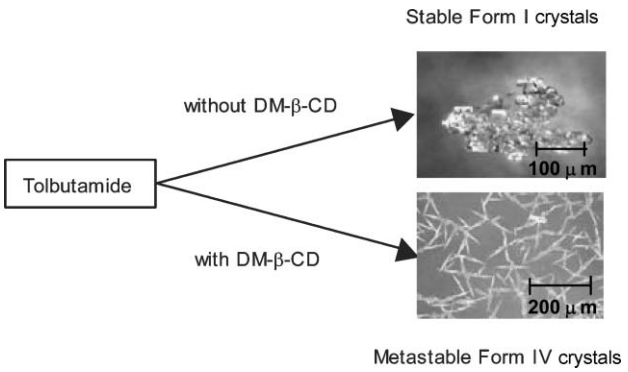



**Fig. 2** Form I and Form IV crystals of tolbutamide precipitated in the absence and presence of DM-β-CD (5 mM) in pH 6.8 sodium phosphate buffer at 4 °C.

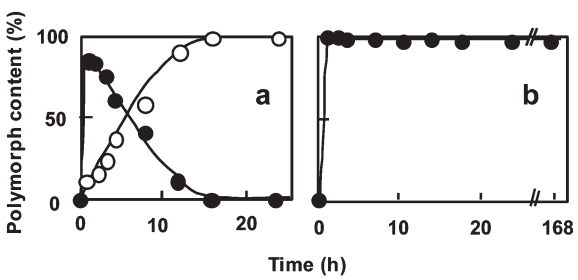



**Fig. 3** Time courses for the appearance and disappearance of tolbutamide polymorphs Forms I (○) and IV (●) in (a) the absence of CDs and (b) the presence of DM-β-CD (5 mM) in pH 6.8 sodium phosphate buffer at 4 °C.

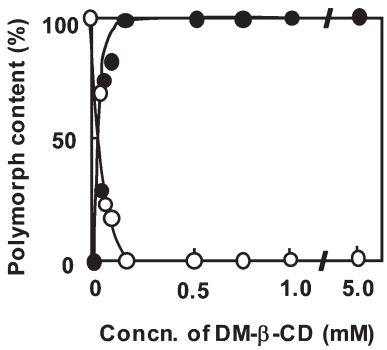



**Fig. 4** Contents of Forms I (○) and IV (●) tolbutamide crystals precipitated in the presence of different DM-β-CD concentrations (0.0–5.0 mM) in pH 6.8 sodium phosphate buffer after storage for 1 day at 4 °C.

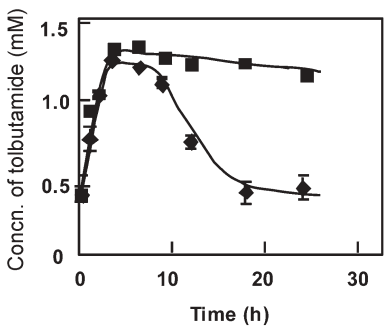
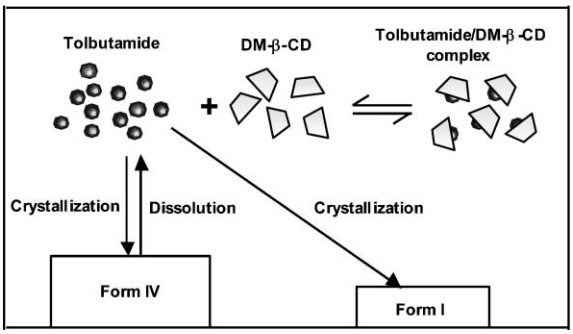



**Fig. 5** Changes in tolbutamide concentration in solution during crystallization at 4 °C, when an excess amount (15 mg) of Form IV crystals was added to a saturated solution of Form I (0.4 mM, 20 mL) in the absence of CDs (◆) and presence of DM-β-CD (■, 5 mM) in pH 6.8 sodium phosphate buffer at 4 °C.




**Scheme 1** One of the possible mechanisms for the inhibition of the solution-mediated polymorphic transition of tolbutamide Form IV to Form I crystals in the presence of DM-β-CD.

The interaction studies using the solubility method[6] and $^1$H-NMR spectroscopy indicated that DM-β-CD forms a 1 : 1 inclusion complex by including the toluene moiety of tolbutamide in its cavity. The stability constants of the complexes determined by the solubility method were 24 $\pm$ 4 $M^{-1}$ (α-CD), 73 $\pm$ 9 $M^{-1}$ (HP-α-CD), 125 $\pm$ 7 $M^{-1}$ (DM-α-CD), 344 $\pm$ 12 $M^{-1}$ (β-CD), 194 $\pm$ 17 $M^{-1}$ (HP-β-CD) and 1920 $\pm$ 32 $M^{-1}$ (DM-β-CD). These results indicate that DM-β-CD strongly interacts with tolbutamide, compared with other CDs employed.

Scheme 1 shows one of possible mechanisms for the selective crystallization of tolbutamide Form IV crystals in DM-β-CD solutions. In the absence of CDs, Form IV crystals with a higher solubility precipitate fast from the solution according to Ostwald's rule. However, once Form I crystal nuclei are formed, even in small amounts, the tolbutamide solution becomes supersaturated with respect to Form I crystals, and therefore Form I nuclei rapidly grow and Form IV crystals dissolve. In the presence of DM-β-CD, on the other hand, an additional equilibrium of inclusion complexation is introduced to the system, and this equilibrium may compete with the crystallization and inhibit the crystallization of Form I. The newly introduced inclusion equilibrium may work as a buffer system that prevents nucleation and crystal growth processes, although further studies have to be done on the nucleation of the stable and metastable crystals, the structural elucidation of the metastable Form IV crystals, and the interaction between the crystal surfaces, the drug and the CDs. However, the present study indicates that DM-β-CD complexation is useful for the stabilization and isolation of Ostwald's metastable forms that occur at an early stage of tolbutamide crystallization. The method described here for the control of polymorphic transformation by CD complex formation will provide an opportunity to isolate labile intermediate metastable polymorphs and will be a valuable tool for the detection and preparation of new polymorphs that are undiscovered so far.

## Notes and references

1 (*a*) S. R. Byrn, *Solid-state Chemistry of Drugs*, Academic Press, New York, 1982; (*b*) *Polymorphism in Pharmaceutical Solids*, ed. H. G. Brittain, Marcel Dekker, New York, 1999; (*c*) J. Bernstein, *Polymorphism in Molecular Crystals*, Oxford University Press, Oxford, 2002.
2 (*a*) R. J. Davey, P. T. Cardew, D. McEwan and D. E. Sadler, *J. Cryst. Growth*, 1986, **79**, 648; (*b*) J. S. Tse, D. D. Klug, J. Ripmeester, A. S. Desgreniers and K. L. Lagarec, *Nature*, 1994, **369**, 724.
3 W. Z. Ostwald, *Physik. Chem.*, 1897, **22**, 289.
4 (*a*) K. Uekama and F. Hirayama, in *The Practice of Medicinal Chemistry*, ed. C. G. Wermuth, Academic Press, Amsterdam, 2003, p. 649–673; (*b*) W. Saenger, J. Jacob, K. Gesseler, T. Steiner, D. Hoffmann, H. Sanbe, K. Koizumi, S. Smith and M. Takaha, *Chem. Rev.*, 1998, **98**, 1787; (*c*) K. Uekama, F. Hirayama and T. Irie, *Chem. Rev.*, 1998, **98**, 2045; (*d*) *Comprehensive Supramolecular Chemistry*, ed. J. Szejtli and T. Osa, Pergamon Press, Oxford, 1996.
5 (*a*) K. Kimura, F. Hirayama and K. Uekama, *J. Pharm. Sci.*, 1999, **88**, 385; (*b*) L. Borka, *Pharm. Acta Helv.*, 1991, **66**, 16.
6 T. Higuchi and K. A. Connors, *Adv. Anal. Chem. Instrum.*, 1965, **4**, 117.