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Cyclodextrin complexes of the anticonvulsant agent valproic acid†

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Six cyclodextrin (CD) complexes of the antiepileptic drug valproic acid (VAL) were prepared by kneading and/or co-precipitation methods and characterized by thermal analysis, powder X-ray diffraction and spectroscopic (^{1}H NMR and FT-IR) techniques. The complexes (with host–guest stoichiometries in parentheses) included α-CD·VAL (2 : 1), β-CD·VAL (1 : 1), γ-CD·VAL (3 : 4), DMB·VAL (1 : 1), TMB·VAL (1 : 1) and TMA·VAL (1 : 1). Single crystal X-ray structures of four of the complexes were determined, those with β-CD and γ-CD featuring severely disordered guest molecules. Instead, the VAL molecules in the complexes with dimethylated β-CD (DMB) and permethylated α-CD (TMA) could be modelled, revealing modes of inclusion of valproic acid in CDs for the first time. The aqueous solubility values at 27 °C for VAL in the form of the solid complexes α-CD·VAL, β-CD·VAL and γ-CD·VAL were in the range 0.26–0.58 times that of the pure liquid phase of VAL. The merits of CD inclusion of VAL (*e.g.* transformation of the liquid guest into a solid, potential for reduction of adverse side effects) are discussed.

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Introduction

Cyclodextrins (CDs) are widely recognized as highly versatile host compounds, with an extraordinary variety of applications in host–guest chemistry, including pharmaceutical development, enantio-separations, food and cosmetics technology, biotechnology, catalysis, nanotechnology and agrochemistry.[1] In a recent editorial entitled "Superstructures with cyclodextrins: Chemistry and applications II", the guest editor affirmed this view, maintaining that "CDs are now considered to be the most interesting class of organic host molecules".[2] the utility of CDs is based on their macrocyclic structures which enable them to include lipophilic guest molecules, wholly or partially, within their hydrophobic cavities through non-covalent interactions, while peripherally located hydrophilic groups render the resulting complex soluble in aqueous media. Derivatisation *via* selective reactions (*e.g.*, alkylation, acetylation, sulfation, *etc.*) on the hydroxyl groups of parent CDs is another distinct advantage of this compound class, affording an extremely wide variety of product CDs that are customised for specific functions.[1] A very recent comprehensive review,[3] celebrating the 130th anniversary of the discovery of these macrocyclic oligosaccharides by Villiers in 1891, indicates that the current volume of literature featuring CDs exceeds 2000 publications annually.

In the pharmaceutical context, CDs have been used primarily for the solubilisation of poorly soluble active pharmaceutical ingredients (APIs) to increase their apparent solubility and enhance their biovailability.[4–6] During the course of several decades, many additional advantages of CD-drug inclusion have been discovered, among them the elimination of unfavourable API taste and odour, increased chemical stability of APIs which are sensitive to light and heat in their uncomplexed state, and the entrapment of volatile APIs.[3] These advantages are exploited in the ongoing use of CDs as carriers in drug-delivery. Less well-known features of certain CDs include their direct use as APIs, rather than as carriers, in treatments of numerous disease states, as described in recent reviews.[7,8]

The present report focuses on the use of selected CDs (Scheme 1) as potential hosts for complexation with an API, namely the antiepileptic drug valproic acid (2-propylpentanoic acid) (Scheme 1).

Of the CDs listed above, the parent compounds α-CD, β-CD, γ-CD as well as DMB [(2,6-di-*O*-methyl)-β-CD] have been approved in specific medicinal preparations,[8] but TMB (permethylated β-CD) and TMA (permethylated α-CD) have not. The latter two have, however, been included in the present study as model host compounds which are known to encapsulate numerous organic guest molecules.

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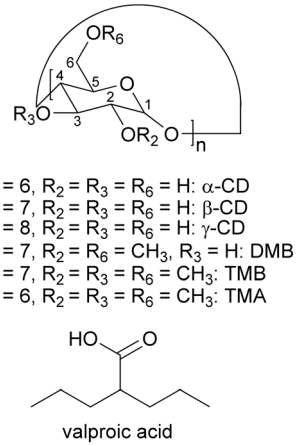

Scheme 1 Chemical structures of (top) the CD hosts employed in the study, and (bottom) the API valproic acid.

A recent review focusing on the mechanism of action and clinical aspects of valproic acid in its therapeutic role in the treatment of epilepsy[9] confirms that it has to date retained its status as one of the most widely prescribed antiepileptic drugs. Interestingly, following its synthesis by Burton in 1882, the acid (a liquid with a yellow tinge under ambient conditions) was employed as a common solvent for compounds being screened for their possible anticonvulsant activity, and remarkably, only in 1963 was valproic acid itself serendipitously found to display the desired activity.[9,10] The drug is used to treat numerous conditions, including various types of seizure, mania in patients with bipolar disorder, and in the prevention of migraine.[11] Its mechanism of action is complex and involves multiple pathways, many of which are poorly understood, but one notable effect is its ability to increase the concentration of γ-aminobutyric acid (GABA) in the brain, resulting in this neurotransmitter blocking nerve transmission in the central nervous system, thereby promoting a calming effect. Many dosage forms of the drug are available, including tablets (delayed release, film coated) and capsules (containing solid or liquid) for oral use, as well as solutions for intravenous administration,[12] these preparations containing either sodium valproate or a mixture of valproic acid and sodium valproate ('valproate semisodium'), and they generally all achieve 90–100% drug bioavailability.[9,12] There are, however, several unfavourable aspects of antiepileptic therapy with valproate/valproic acid. A wide range of adverse side effects may arise, depending on patient profiles such as age, gender, and general medical condition. Recent articles and medical sources detail known possible side effects, namely hepatic, neurological, dermatological and other disorders, the most common, however, being pre-absorption gastrointestinal disturbances,[9,13,14] characterized by *e.g.*, abdominal pain, dyspepsia, nausea, vomiting, gastroenteritis, ranging up to pancreatitis. This is the rationale for prescribing delayed-release drug forms which prevent damage to the stomach by releasing the drug in the intestine.

The aim of the present study was to test the feasibility of forming CD inclusion complexes between the series of selected CDs (Scheme 1) and the API valproic acid (VAL). This was motivated by several considerations, namely (a) the possible advantage of incorporating the liquid API into a new solid phase without compromising its bioactivity, (b) the potential for reducing the most common adverse side effect of oral administration of valproic acid, namely gastrointestinal disorders, by masking the acidic function of the API through its encapsulation within the cavities of CD molecules, (c) providing CD–VAL complexes that might display different pharmacokinetic behaviour from that of existing VAL formulations. To elaborate on consideration (b) above, this was based on the known ability of CD complexes of certain non-steroidal anti-inflammatory drugs (NSAIDs) to reduce gastrointestinal irritation that results when the untreated NSAIDs are administered orally.[6,15] Consideration (c) is likewise a realistic one, given that oral administration of CD complexes of several NSAIDs has also led to more rapid onset of API action compared to the untreated drugs (as exemplified by the highly successful performance of the β-CD complex of piroxicam, whose safety and efficacy as a medication are well established).[15] A further point to note is that CD-inclusion of valproic acid could eliminate the need to use sodium valproate, whose hygroscopicity is a complicating factor in pharmaceutical processing and storage[16,17] and whose cost is much greater than that of valproic acid.[18] It is important to state here that while the motivation for this study is based on the various considerations listed above, the present report is confined to the question of the feasibility of preparing and characterizing CD inclusion complexes of valproic acid. As detailed in the next section, we present our results that reflect successful isolation of six CD–VAL complexes and their physicochemical characterization using thermal analysis, X-ray diffraction and spectroscopic methods.

A literature search for crystalline products of CD–VAL complexation indicated that relatively little research on this topic has been performed to date. A study of the complexation of VAL using the amorphous CDs hydroxypropyl-β-CD (HP-β-CD) and sulfobutylether-β-CD (SBE-β-CD), as well as solid dispersions of VAL in polymers (PEG polyethylene glycol 6000 and polyvinylpyrrolidone K-30), yielded the expected amorphous products. Improvement in VAL solubility was much more significant with HP-β-CD as host than with SBE-β-CD.[19] The report also refers to two earlier (1981, 1998) studies of complex preparation involving β-CD, both of which, however, involved the sodium salt of VAL as the API, which is not relevant here. A search of the Cambridge Crystallographic Database (CSD)[20] for crystals containing valproic acid yielded two hits. The first is trisodium hydrogentetravalproate monohydrate $[Na_3(valproate)_3(valproic\ acid)\cdot H_2O]$[21] (refcode SITXID). The accidental occurrence of the free acid VAL in this structure was attributed to partial hydrolysis of the valproate ion. The second VAL-containing compound deposited in the CSD is

the very recently reported ionic co-crystal lamotriginium valproate lamotrigine tris(valproic acid) (refcode ROSYIK),[22] the coformer lamotrigine (LAM) being another well-known antiepileptic drug. The authors state that both the dissolution rate and the tabletability of this compound were improved when compared with the performance of pure LAM. Finally, with relevance to the present report, we emphasise that the search of the CSD mentioned above did not reveal any crystal structures of CD complexes of VAL.

Experimental

Valproic acid (2-propylpentanoic acid, VAL; $C_8H_{16}O_2$) was purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). The host compounds β-cyclodextrin (β-CD; $C_{42}H_{70}O_{35}$), hexakis(2,3,6-tri-*O*-methyl)-α-cyclodextrin (permethylated α-CD, TMA; $C_{54}H_{96}O_{30}$), heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (dimethyl-β-CD, DMB; $C_{56}H_{96}O_{35}$), heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (permethylated β-CD, TMB; $C_{63}H_{112}O_{35}$), randomly methylated β-cyclodextrin (RAMEB; $(C_{42}H_{70\text{-}n}O_{35})(CH_3)_n$, with n = 12) and hydroxypropyl β-cyclodextrin (HP-β-CD; $(C_{42}H_{70\text{-}n}O_{35})(C_3H_7O)_n$, with n = 4.5) were purchased from Cyclolab (Budapest, Hungary). Samples of α-cyclodextrin (α-CD; $C_{36}H_{60}O_{30}$) and γ-cyclodextrin (γ-CD; $C_{48}H_{80}O_{40}$) were acquired from Wacker, Biosolutions (Halle, Germany).

The co-precipitation method was employed to isolate complexes of the three parent CDs with VAL. An accurately weighed amount of each CD was suspended in a measured volume of water in a separate vial, as follows: for α-CD, 67.46 mg (0.0693 mmol) in 0.7 cm^3; for β-CD, 39.35 mg (0.0347 mmol) in 2.2 cm^3; for γ-CD, 59.96 mg (0.0462 mmol) in 2.0 cm^3. The three suspensions were heated to 70 °C with continuous stirring to obtain clear solutions. A mass of 5 mg (0.0347 mmol, 5.434 μl) of valproic acid was added to each solution and the resultant mixtures were magnetically stirred at 70 °C for 2, 6 and 6 h respectively. Each solution was finally filtered (0.45 μm nylon microfilter) and slow cooling followed. The crystalline products were harvested after 4 days. For isolation of complexes of the methylated CDs, the latter were separately dissolved in cold water in the following mass/volume ratios: 46.16 mg (0.0347 mmol) of DMB in 0.6 cm^3; 49.56 mg (0.0347 mmol) of TMB in 0.8 cm^3; 42.48 mg (0.0347 mmol) of TMA in 0.8 cm^3. Following addition of 5 mg (0.0347 mmol; 5.434 μl) of valproic acid to each CD solution, the three resulting mixtures were stirred overnight at 25 °C. Thermal cycling followed; this involved alternate heating and cooling of the solutions in the temperature range 4–70 °C at 10 minute intervals with continuous stirring. After four repetitions of this process, each solution was filtered (0.45 μm nylon microfilter) into a clean vial. The three vials were sealed and incubated at 60 °C to encourage complex crystallization and growth. Single crystals appeared after 3 days. It was also possible to prepare some of the complexes by kneading each CD and VAL mixture having the correct stoichiometric ratio.

Complex formation was initially established using powder X-ray diffraction (PXRD) analysis. Samples of the products of co-precipitation or kneading were finely ground, placed on a zero-background holder and their PXRD patterns were recorded at 21 °C on a D8 Advance instrument with Cu$K\alpha_1$ X-rays (λ = 1.5406 Å). Generator settings were 30 kV and 40 mA, and samples were scanned with step size 0.0164° over the 2θ-range 4–40°. Approximate unit cell dimensions and space groups were generally successfully predicted by comparing the experimental patterns with published computed reference patterns for known isostructural series of CD complexes.[23]

Host–guest stoichiometries were generally determined by dissolving crystals of the inclusion complexes in DMSO-d_6 and recording the ^{1}H nuclear magnetic resonance (NMR) spectra of the solutions at 298 K on a Bruker Ultrashield 400 Plus spectrometer. Spectral analysis was performed with the program MestReNova.[24] Thermal analysis of complex crystals included hot stage microscopy (HSM), thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). For HSM studies, a Linkam THM600 instrument with a TP92 temperature control unit was employed, with samples heated at a constant rate of 10 K min^{-1} in the range ~20–400 °C. Thermal events were monitored using a Nikon SMZ-10 stereo microscope and images were recorded on a Sony Digital Hyper HAD colour video camera and viewed with the Soft Imaging Program AnalySIS.[25] DSC curves were recorded on a DSC XP-10 instrument (THASS: Thermal Analysis & Surface Solutions GmbH, Friedberg, Germany) with dry N_2 purge gas flowing at a rate of 60 cm^3 min^{-1} and a sample heating rate of 10 K min^{-1} to a maximum value of 250 °C. Data were analysed with the software package Differential Scanning Calorimeter, version 3.3.0.7 (D).[26] Thermogravimetry was performed on a TA-Q500 apparatus (Texas Instruments) controlled with Universal Analysis 2000 software.[27] Complex samples with mass range 0.9–3.5 mg were rapidly dried on filter paper to absorb surface water and then placed in an alumina crucible for analysis at a heating rate of 10 K min^{-1} to a maximum temperature of 400 °C. From the combined NMR data and TGA data for complex dehydration, it was possible to determine the complete stoichiometric formula $(CD)_p{\cdot}(VAL)_q{\cdot}(H_2O)_r$ of each ternary complex.

Single-crystal X-ray intensity data were collected on a Bruker Kappa APEX II Duo diffractometer with crystals mounted on nylon cryoloops and coated with paratone N oil (Exxon Chemical Co., TX, USA). Unit cell dimensions were generally recorded at 294 K initially and the crystals were subsequently cooled to the desired constant low temperature using a Cryostream cooler (Oxford Cryosystems, Oxford, UK). The structures were solved by isomorphous replacement or direct methods and refined by full-matrix least-squares using the SHELX program suite[28] implemented in the X-SEED interface.[29] Non-hydrogen atoms were generally refined anisotropically and H atoms were included in a riding model with U_{iso} values in the range 1.2–1.5 times those of their parent atoms. Oxygen atoms of water molecules were treated

anisotropically only if they had been assigned full occupancy, and no water H atoms were included in the models. Non-routine details of refinement are provided with the descriptions of individual structures and additional programs employed in the analyses are listed in the CIF files.

Fourier transform infrared (FTIR) spectra were recorded on a PerkinElmer Spectrum Two instrument using the attenuated total reflectance (ATR) technique with a scan range of 4000–400 cm^{-1} with maximum resolution 0.5 cm^{-1}.

Solubility measurements with pure water as the medium were performed to establish the effect of inclusion of VAL in the α-, β- and γ-CD complexes on the solubility of the API, relative to that of pure VAL at room temperature, namely 1.3 mg cm^{-3}.[30] VAL is known to have a weak UV absorbance at less than 200 nm,[31] hence a gravimetric method was used instead. Duplicate solubility measurements were conducted simultaneously for each of the three inclusion complexes. The method involved the addition of small pre-weighed incremental amounts of each CD·VAL inclusion complex into a vial containing 1.0 cm^3 of Milli-Q water (Millipore corporation, Billerica, Massachusetts, USA). A visual estimation of the solubility was established after the final suspension was stirred for 72 hours at 27 °C. The results were recorded as the range between the penultimate and final masses of CD complex added, the latter amount resulting in the first appearance of precipitation. The experiment was performed on a Radleys standard stirring hotplate (Radleys, Essex, UK) with an attached vial-supporting stand. The vials were placed on the stand in a circle at a radius of 2.0 cm from the centre. The stirring rate was set to 250 rpm at a temperature of 27 °C.

Results and discussion

The morphologies of the six inclusion complexes are shown in Fig. 1. Co-precipitation experiments yielded single crystals of β-CD·VAL, γ-CD·VAL, DMB·VAL and TMA·VAL. Instead, α-CD·VAL occurred as clusters of fine whiskers and TMB·VAL formed arbitrarily shaped lamellae. Recrystallization of the α-CD·VAL complex from water, DMSO, ethylene glycol and pyridine did not alter the morphology or crystal size.

The host–guest stoichiometries of the complexes were determined unequivocally from their solution-state ^{1}H NMR spectra (ESI,† Fig. S1–S6, Tables S1–S6). For the VAL complexes with the parent hosts, the results were: α-CD·VAL 2 : 1; β-CD·VAL 1 : 1; γ-CD·VAL 3 : 4. The three complexes with the methylated CD hosts, DMB·VAL, TMB·VAL and TMA·VAL were all found to have 1 : 1 host–guest stoichiometry.

The main purpose of the thermal analysis studies was to determine the water contents of each complex. Fig. 2 is a representative TGA-DSC profile for the α-CD·VAL complex. The profiles for all the complexes are provided (ESI,† Fig. S7–S12) and the derived DSC data (onset and peak temperatures of thermal events) and TGA data (percentage mass losses and calculated numbers of water molecules per CD complex unit) are available (ESI,† Table S7).

The TGA trace for α-CD·VAL (Fig. 2), and those for β-CD·VAL, γ-CD·VAL and DMB·VAL displayed two distinct mass losses, the first being associated with complex dehydration and the second with eventual decomposition of the anhydrous complex. However, the TGA traces for the remaining complexes displayed different features, namely loss of the guest, valproic acid, followed by host decomposition for TMB·VAL, and in the case of TMA·VAL, mass losses due to dehydration, a two-step guest loss and host decomposition. The two-step loss involved a large mass loss followed by a small, very gradual one. The reported experimental percentage guest losses from the TGA data for these two complexes (ESI,† Table S7) are in accord with the percentages calculated from the final complex formulae based on the ^{1}H NMR spectra and water mass losses, as follows: for TMB·VAL, 8.3 ± 0.9% *vs.* 9.2%, and for TMA·VAL 9.9 ± 0.7% *vs.* 10.4%. DSC curves were also informative, indicating stepwise water loss for α-CD·VAL, β-CD·VAL and TMA·VAL, and for the latter complex, following guest loss, fusion of the host TMA at 221 °C (previously reported as 217.6 °C).[32] Both γ-CD·VAL and DMB·VAL displayed single-step dehydration, while the DSC curve of TMB·VAL showed a single sharp endotherm, attributed to the fusion of TMB at 131 °C (previously reported melting points for different polymorphs 148 and 157 °C).[33]

Fourier transform infrared (FT-IR) spectra were recorded for all six complexes to determine the possible effect that CD inclusion might have on the carbonyl stretching frequency of

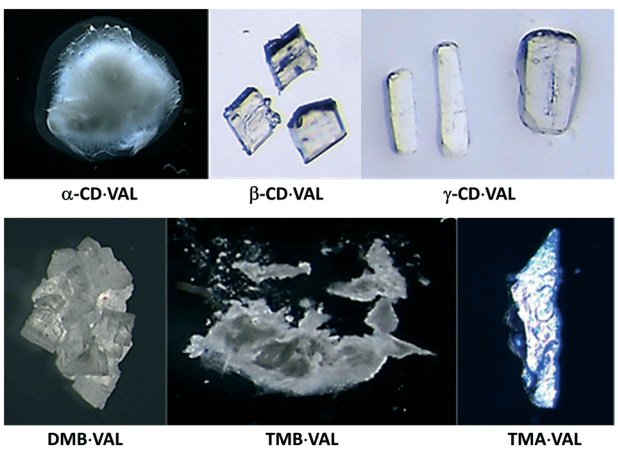

Fig. 1 The morphological features of the six CD complexes of valproic acid.

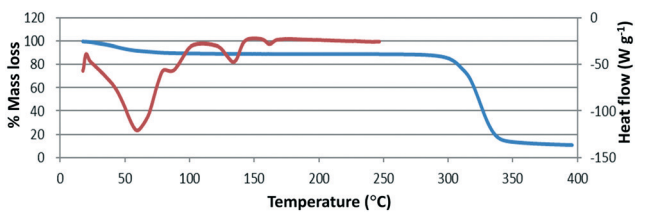

Fig. 2 Representative DSC (red) and TGA (blue) curves for α-CD·VAL.

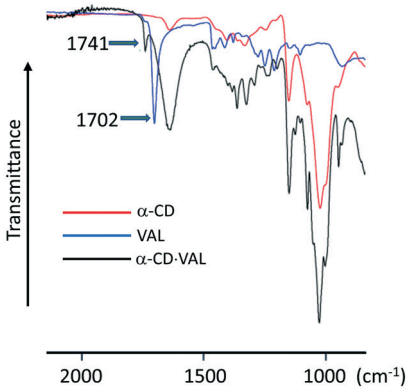

Fig. 3 The shift of ν(C═O) of valproic acid on its inclusion in α-CD.

valproic acid (1702 cm^{-1}). A representative example is shown in Fig. 3, where ν(C═O) increased very significantly ($\Delta\nu$ = 39 cm^{-1}) on inclusion of VAL in α-CD. Analogous shifts to higher wavenumbers occurred for the inclusion of VAL in DMB, TMB and TMA, the new stretching frequencies appearing at 1731, 1726 and 1734 cm^{-1} respectively (ESI,† Fig. S13–S18), and these increases reflecting different extents of interaction of VAL within the new micro-environments of the respective CD cavities. In the complexes β-CD·VAL and γ-CD·VAL, the ν(C═O) values were 1704 and 1702 cm^{-1} respectively. Thus, the widely-ranging characteristic ν(C═O) values listed above can in principle serve as a means of complex identification for each member of the series investigated. It is interesting to note, however, that ν(C═O) for pure VAL was unchanged in the reported complexes of VAL with hydroxypropyl-β-CD and sulfobutylether-β-CD as hosts and only a reduction in band intensity was evident.[19]

Preliminary powder X-ray diffraction (PXRD) patterns for the six products were distinctly different from the sums of the patterns of the respective hosts and VAL, confirming the formation of new crystalline phases. PXRD analyses also revealed that each of the four complexes α-CD·VAL, β-CD·VAL, γ-CD·VAL and TMA·VAL could be prepared by both kneading and co-precipitation methods. A representative example, that for α-CD·VAL, is shown in Fig. 4 and the analogous patterns for the other three complexes are also available (ESI,† Fig. S19–S22).

For α-CD·VAL and TMB·VAL, which were not amenable to single-crystal X-ray analysis, it was of interest to determine whether either of these phases had known isostructural analogues. In the case of α-CD·VAL, its PXRD pattern did not match any of those for common isostructural series of α-CD complexes.[20,23]

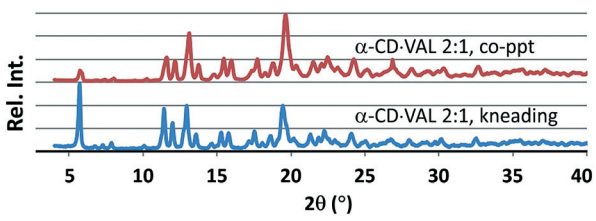

Fig. 4 PXRD patterns for the complex α-CD·VAL prepared by two methods.

The PXRD pattern of the product obtained by co-precipitation of TMB and VAL showed some resemblance to that calculated for TMB·methylcyclohexane (CSD refcode XAQJII)[20] (ESI,† Fig. S23). However, it was of interest to note that the product of kneading TMB and VAL yielded a different PXRD pattern. The latter correlated fairly well with that for known TMB complexes belonging to the isostructural series crystallizing in the space group $P2_12_12_1$ with approximate unit cell dimensions $a \approx 15$ Å, $b \approx 21$ Å, $c \approx 28$ Å,[23] of which the TMB·(4-biphenylacetic acid) complex (refcode PAFSOE) is a member (ESI,† Fig. S24). The new phase obtained by kneading did not, however, yield single crystals on recrystallization and was therefore not pursued further.

The remaining four complexes, β-CD·VAL, γ-CD·VAL, DMB·VAL and TMA·VAL were thus candidates for analysis by SCXRD and their structures are described below. Crystal data and refinement details are available (ESI,† Table S8). It was evident from the space group and unit cell dimensions of β-CD·VAL [moiety formula $[(\beta\text{-CD})_2\cdot(\text{VAL})_2\cdot 9.2H_2O]$, that this complex belongs to the well-known isostructural series of β-CD complexes crystallizing in the space group C2, in which the asymmetric unit (ASU) contains one unique β-CD molecule which forms a dimer with C_2 symmetry (Fig. 5a) *via* O–H···O hydrogen bonding between the hydroxyl groups on the wider secondary rims of the CDs. A characteristic of this

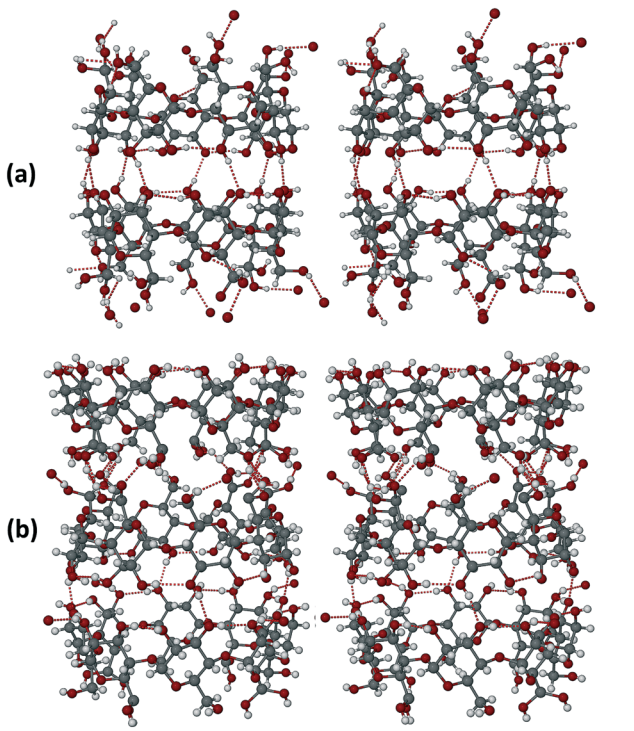

Fig. 5 Stereoscopic views of the dimeric motif in the β-CD·VAL complex (a) and the motif comprising three unique host molecules occurring in the γ-CD·VAL complex (b), showing the intramolecular and intermolecular O–H···O hydrogen bonds. Water molecules have been omitted for clarity.

isostructural series is the frequent occurrence of guest disorder that may, in special circumstances, be amenable to modelling. However, the extent of the disorder frequently results in little or no electron density appearing within the 'cage' formed by the union of the two CD cavities. The disorder is attributed to aperiodic location of guest molecules within the continuous channel of overlapping cages generated *via* head-to-tail stacking of the dimers. This is the case for β-CD·VAL, whose final structural model includes the host dimer and its complement of water molecules. However, despite the extreme guest disorder, the chemical composition of the hydrated crystal is based on sound thermal and NMR spectroscopic data and is thus well-defined.

Similar remarks apply to the γ-CD·VAL complex (Fig. 5b), with moiety formula $(\gamma\text{-CD})_3\cdot(\text{VAL})_4\cdot 49H_2O$, crystallizing in the tetragonal space group $P42_12$, with three independent γ-CD molecules in the unit cell. These molecules are all located on a common tetrad, forming infinite channels by head-to-tail, head-to-head and tail-to-tail stacking. The valproic acid molecules are fully disordered within the channels. Again, only rarely is it possible to locate ordered guest molecules in the channel, a well-known example being that of the complex γ-CD 12-crown-4 nonahydrate (CSD refcode DOCYID),[20] whose guest molecule satisfies the required fourfold symmetry of the γ-CD cavity. The packing arrangements of both β-CD·VAL and γ-CD·VAL feature infinite columns of CD complexes separated from one another by channels accommodating water molecules. The two isostructural series to which these complexes belong are well represented in both the CSD[20] and the literature on cyclodextrin complexes, and therefore no further discussion of their structural features is warranted here. From the viewpoint of potential pharmaceutical delivery of valproic acid, both complexes could be considered as candidates, γ-CD complexes being more desirable, however, owing to the low toxicity of the host compound.[34]

Of the four CD-VAL complexes amenable to SCXRD analysis, only DMB·VAL and TMA·VAL revealed, for the first time, modes of encapsulation of valproic acid guest molecules within the cavities of CD molecules. Solution of the structure of the DMB complex (space group *P*1) revealed two host molecules (A and B) in the ASU, molecule A having the expected formula for DMB, with all seven rings showing 2,6-di-*O*-methylation (Scheme 1), but molecule B differing in having one of the seven glucose rings 2,3,6-tri-*O*-methylated. This is a small aberration, accounted for in assigning an accurate complex moiety formula (ESI,† Table S8). This phenomenon of partial overmethylation of DMB affecting only one glucose ring has been observed previously in the crystal structures of the DMB complexes of 2-methoxyestradiol[35] and naringenin.[36] For simplicity, however, here we refer to the host molecules in the two independent complex units as DMB(A) and DMB(B). Due to the abnormally high thermal motion of the included VAL molecules, their free refinement was unstable and it was necessary to add distance restraints (mean values taken from the structure of the co-crystal ROSYIK[22]) and angle restraints, and to assign two global variable U_{iso} parameters to both VAL molecules, which refined to 0.222(4) and 0.230(5) Å^2. Fig. 6 shows the structure of the complex, the general view being into the (wider) secondary side of each DMB molecule to highlight the modes of guest inclusion in the two independent complex molecules labelled A and B. Molecule DMB(A) displays the well-known intramolecular 'belt' of seven homodromic H-bonds O3(n)–H···O2(n + 1) that link contiguous glucose units, accounting for the relatively undistorted shape of the host molecule. For host molecule DMB(B), however, there are only six H-bonds of this type, the 'belt' being interrupted at the glucose unit whose O3 atom is methylated.

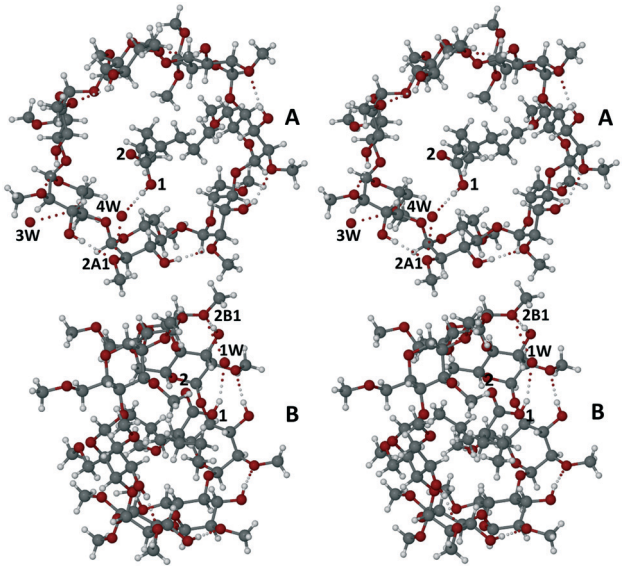

Fig. 6 Stereoscopic view of the two independent complex units A and B in DMB·VAL showing principal hydrogen bonds.

The most important features, however, are the H-bonds involving the guest molecules. In each case the hydrocarbon chain of the VAL molecule spans the length of the host cavity while its –OH group is H-bonded to a water molecule which, in turn, is H-bonded to an O2 atom of DMB. Such mediation of guest inclusion in CDs *via* a bridging water molecule is not unusual. We note, in particular that the C═O groups of the VAL molecules shown in Fig. 6 make no significant intermolecular contacts. This observation is consistent with the FTIR results which indicated a value of 1731 cm^{-1} for ν(C═O) of VAL in the complex DMB·VAL, implying a stronger carbonyl bond than that occurring in the VAL pure liquid phase (ν(C═O) = 1702 cm^{-1}) in which there is undoubtedly a significant level of molecular association *via* H-bonding. Details of the H-bonding relating to guest inclusion (Fig. 6) are as follows: complex A, VAL(O1A–H)···O4W–H···O2A1(DMB), with the distances O1A···O4W 2.78(3) Å and O4W···O2A1 2.98(2) Å; for complex B, the corresponding data are O1B···O1W 3.01(3) Å and O1W···O2B1 2.96(1) Å. Fig. 7 shows cutaway space-filling images of complex units A and B,

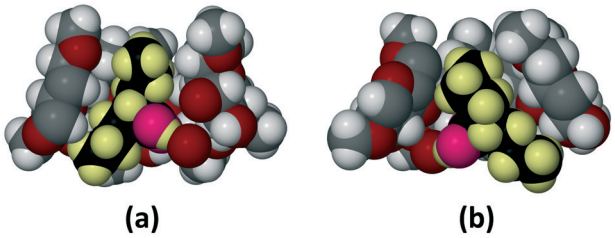

Fig. 7 Cutaway views showing the guest inclusion mode in complex units A (a) and B (b) in the crystal of DMB·VAL. For clarity, the colour scheme for molecules of valproic acid is different from that for the host molecules.

the common –COOH···O(water oxygen) hydrogen bond being visible in both structures. Also evident is the close fitting of the hydrocarbon chains to the hydrophobic cavity surfaces of the host molecules.

A detailed analysis of the conformations of host molecules A and B of DMB·VAL was performed by evaluating a frequently used set of structural parameters whose values indicate deviations from ideal seven-fold symmetry. The atomic numbering schemes as well as the numbering of the individual glucose residues are provided (ESI,† Fig. S25 and S26) and the definitions and values of these parameters are listed (ESI,† Tables S9 and S10). One notable indicator of asymmetry is the tilt angle (τ_2), defined as the angle between the mean plane O4n–C1n···C4n–O4(n + 1) (*i.e.*, the 'backbone' of the n^{th} glucose residue) and the mean plane of the O4-heptagon (comprising the seven glycosidic oxygen atoms). The τ_2 ranges are 4.2–22.7° for host DMB(A) and 3.6–27.5° for DMB(B). There is a general correlation between the tilt angles for the corresponding glucose residues of the two hosts, but the major conformational difference is that residue B3 in DMB(B) has a τ_2 value of 27.5°, whereas for residue A3 in DMB(A), τ_2 is only 19.2°. This distortion in DMB(B) results from steric repulsion between residues B2 and B3 (the latter being the 'overmethylated' residue), resulting in a short contact C8B2···O2B3 of 3.03(3) Å. The hydrocarbon chain conformations of the included VAL molecules differ somewhat (all geometrical parameters appear in the CIF files) but in both cases, as is evident from Fig. 6, the plane of each –COOH group is approximately perpendicular to the mean plane through the respective hydrocarbon chain.

The complex TMA·VAL crystallizes in the orthorhombic space group $P2_12_12_1$ and it also has 1:1 host–guest stoichiometry, the ASU consisting of one TMA molecule, one VAL molecule and nominally 1.2 water molecules. In contrast to the situation in the DMB complex, where abnormally high thermal motion of the guest molecules was evident, the U_{iso} values for the carbon atoms of the hydrocarbon chain of the VAL molecule averaged only ~0.06 Å^2 and they could subsequently be refined anisotropically. However, the carboxylic acid group was found to be disordered over two orientations, with refined site-occupancy factors (s.o.f.s) 0.731(4) and 0.269(4), and the common free variable for U_{iso} of the atoms of the O–C═O components refined to 0.058 Å^2

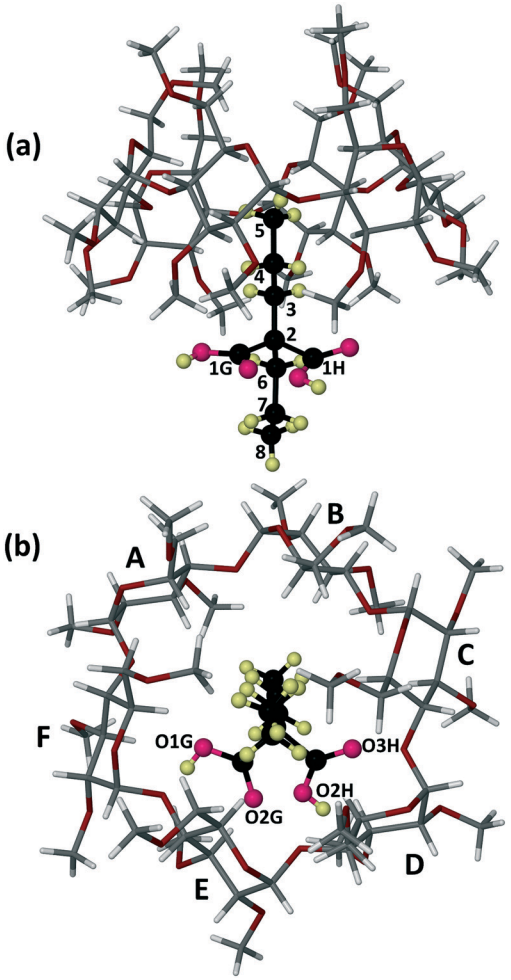

Fig. 8 Perspective views of TMA·VAL from the side of the TMA molecule (primary rim above, secondary rim below) (a), and from the primary rim of the TMA molecule (b). In (a), the numerical labels of the carbon atoms of the valproic acid molecule are shown. In (b), the six glucose residues are labelled A–F and the labels for the oxygen atoms of the disordered –COOH groups are shown.

(*i.e.*, a very similar value to those of the hydrocarbon chain atoms). In Fig. 8(a), the TMA molecule is viewed from the side, with its primary (narrow) rim at the top and the (wider) secondary rim below. Only one propyl group of the valproic acid molecule is included within the TMA cavity due to the partial 'capping' of the host molecule by bulky methoxymethyl substituents on the primary rim (evident in Fig. 8(b), which is a view of the complex from the primary rim). The components of the disordered carboxylic acid group (major component on the left side of both figures) and the rest of the linear hydrocarbon chain protrude from the secondary side, into a pocket formed at the junction of three neighbouring complex units (ESI,† stereoscopic Fig. S27). This mode of guest inclusion, with part of the guest molecule included in a CD cavity and the remaining residue nestling in a void created by the juxtaposition of neighbouring CD molecules, has been observed previously for a steroid in a methylated CD.[37]

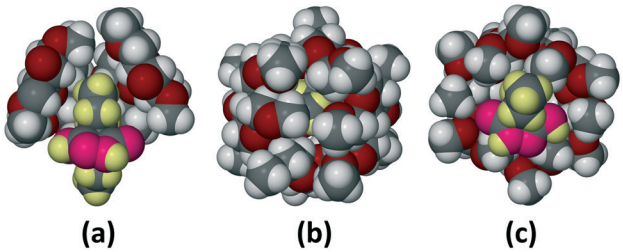

Fig. 9 Space-filling perspective images of TMA·VAL: cutaway view showing the inclusion of one of the propyl groups of valproic acid in the CD cavity (a); view of the complex from the primary rim, showing the 'capping' of the CD by methoxymethyl groups (b); view from the secondary rim of the TMA molecule showing the protruding residue, including the disordered carboxylic acid group (c).

Space-filling views of the TMA·VAL complex are shown in Fig. 9, supporting the description above. In addition, the detailed conformational parameters for the TMA molecule are provided (ESI,† Table S11).

For interest, we performed a gravimetric study (method described earlier in this report) to determine the effect of the inclusion of VAL in the three parent CDs on the aqueous solubility of this API. The measured data for the solubility of VAL based on the dissolution of the three pure complexes (ESI,† calculations: gravimetric solubility analysis) were compared with the aqueous solubility of pure VAL, namely 1.3 mg cm^{-3}. Calculation of the ratios $S_{VAL(CD)}/S_{VAL}$ ($S_{VAL(CD)}$ being the solubility of VAL in the form of the CD complex and S_{VAL} the solubility of pure liquid VAL) yielded 0.26, 0.40 and 0.58 for α-, β- and γ-CD respectively. These results demonstrate, at the very least, that we are not dealing with insoluble complexes and hence the opportunity exists for the release of VAL from these solid forms in aqueous media *via* the usual dissociation mechanism governing the equilibria of CD complexes. Finally, while valproic acid maintains its status as a widely used first-line antiepileptic drug, a growing interest in its demonstrated potential for the treatment of certain cancers is evident from recent reviews.[19,38] Possible repurposing of valproic acid as a potential therapeutic in preventing severe COVID-19 has also recently been discussed.[39] CD complexes of valproic acid as alternative solid forms may therefore also find utility in these developing therapeutic areas.

Conclusions

Accurate compositions of six crystalline CD inclusion complexes containing the guest API valproic acid and water have been established by a combination of ^{1}H NMR spectroscopy (to determine host–guest stoichiometry) and thermal analysis (for water content). Two of the complexes, α-CD·VAL and TMB·VAL, were not amenable to SCXRD analysis due to their intractable morphologies. For both the β-CD·VAL and γ-CD·VAL crystals, SCXRD revealed their complete host frameworks and complements of ordered and disordered water molecules. However, the severely disordered VAL molecules within the extended channels formed by stacking of the respective host molecules with strict alignment of their cavities prevented their modelling. Instead, both the structures and modes of inclusion of valproic acid molecules in the complexes DMB·VAL (two independent ordered guest molecules) and TMA·VAL (a single guest molecule with a disordered –COOH group) were determined successfully by SCXRD. From a pharmaceutical perspective, the complexes of TMA and TMB are not relevant since these host compounds are not approved for medicinal use. However, α-CD, β-CD, γ-CD and DMB have all been employed in drug delivery to various extents and their complexes with valproic acid therefore have potential for further investigation in this context. In the present study, the complexes α-CD, β-CD and γ-CD were shown *via* a simple solubility test to dissolve in water, providing a slightly reduced concentration of valproic acid relative to that of the uncomplexed API, but of the same order of magnitude. Certainly, the conversion of the liquid API into a solid form *via* its inclusion in CDs would be judged as a significant advantage, given the known challenges arising in the practical handling of liquid APIs. Furthermore, encapsulation of the API molecule within CD cavities could be effective in reducing the most common side effect of valproic acid administration, namely gastrointestinal disorders. Critical questions relevant to drug delivery, such as adequacy of effective dosing with relatively large carrier molecules, API bioavailability and possible beneficial impact following the use of any of the four CD complexes would, however, necessitate further studies using *in vitro* and especially *in vivo* tests.

Author contributions

Conceptualization: M. R. C.; formal analysis: A. I. V., M. R. C.; funding acquisition: M. R. C.; investigation: A. I. V.; methodology: A. I. V.; project administration: M. R. C.; resources: M. R. C.; supervision: M. R. C.; validation: A. I. V.; M. R. C.; visualisation: A. I. V.; M. R. C.; writing – original draft: A. I. V.; writing – reviewing and editing: M. R. C.; A. I. V.

Conflicts of interest

There are no conflicts to declare.

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