

# A chemical preformulation study of a host–guest complex of cucurbit[7]uril and a multinuclear platinum agent for enhanced anticancer drug delivery†

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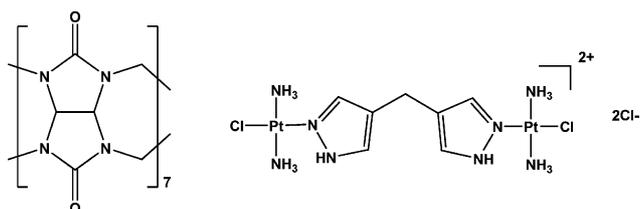
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Single crystal and powder X-ray diffraction have been used to examine the host–guest complex of cucurbit[7]uril (CB[7]) and the model dinuclear platinum anticancer complex *trans*-[PtCl(NH<sub>3</sub>)<sub>2</sub>]<sub>2</sub>μ-dpzm]<sup>2+</sup> (di-Pt, dpzm = 4,4'-dipyrazolylmethane). The single crystal structure shows that the host–guest complex forms with the di-Pt dpzm ligand within the CB[7] cavity and with the platinum groups just beyond the macrocycle portals. Binding is stabilised through hydrophobic interactions and six hydrogen bonds between the platinum ammine ligands and the dpzm pyrazole amine to the CB[7] carbonyls. Each host–guest complex crystallises with two chloride counterions and 5.5 water molecules. The unit cell comprises four asymmetric units, each of which contains three crystallographically independent CB[7]–di-Pt moieties. X-Ray powder diffraction demonstrated structural consistency of the bulk crystals with a single polycrystalline phase that is identical with the single crystal structure. Finally, the effect of CB[7] encapsulation of the thermal stability of di-Pt was examined by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). From the TGA experiments it was found that free CB[7] and the CB[7]–di-Pt complex lose 11 and 3.5% of their mass respectively, through the loss of water molecules, upon heating to 160 °C. The DSC results showed that the free dpzm ligand melts between 186 and 199 °C, with a standard enthalpy of fusion of 27.92 kJ mol<sup>-1</sup>. As a 2+ inorganic salt the metal complex does not melt but undergoes several decomposition events between 140 and 290 °C. Encapsulation by CB[7] completely stabilises di-Pt with no decomposition of either the macrocycle or metal complex at temperatures up to 290 °C.

## Introduction

Cucurbit[*n*]urils (CB[*n*], *n* = 5, 6, 7, 8, or 10) are a family of macrocycles made from the acid catalysed reaction of glycoluril and formaldehyde (Fig. 1).<sup>1</sup> Each CB[*n*] has a hydrophobic cavity within which small molecules can be encapsulated. The resultant host–guest complexes are stabilised by hydrophobic effects between the cavity of the CB[*n*] and the guest, and can be further stabilised by ion–dipole or dipole–dipole interactions between the CB[*n*] carbonyl portals and the guest.<sup>1</sup> Their rigid structure, ease of synthesis and variety of CB[*n*] size and functionality mean they can be used for a variety of applications.<sup>2</sup>

Multinuclear platinum complexes represent a new class of anticancer agent that are able to overcome many forms of cisplatin resistance in *in vitro* and *in vivo* models.<sup>3–6</sup> Whilst these complexes are significantly more active than cisplatin, the standard in platinum-based chemotherapy, they are also considerably more toxic.<sup>5,7,8</sup> BBR3464 is a trinuclear platinum complex that was recently in Phase II clinical trials,<sup>9,10</sup> but has shown little efficacy in humans because of rapid degradation and deactivation.<sup>5</sup> Newer drugs, like the dinuclear complex CT-3610, are also just as



**Fig. 1** The chemical structures of cucurbit[7]uril (CB[7]) and the model dinuclear platinum anticancer complex: *trans*-[PtCl(NH<sub>3</sub>)<sub>2</sub>]<sub>2</sub>μ-dpzm]<sup>2+</sup> (di-Pt).

susceptible to degradation, thus driving the need to develop delivery vehicles for this important class of drugs.

Therefore our group, and other investigators, are particularly interested in the medicinal application of CB[*n*]s as both drug delivery vehicles and in diagnostics/imaging.<sup>11–26</sup> Previously we have reported the use of CB[6], CB[7] and CB[8] as drug delivery vehicles for platinum-based anticancer agents and have proposed three-dimensional host–guest complexes based on molecular modelling.<sup>27–34</sup> In the human body, platinum drugs are easily deactivated and/or degraded by thiol containing proteins and peptides, particularly glutathione.<sup>35</sup> Encapsulation of mononuclear platinum drugs, like cisplatin and oxaliplatin, and multinuclear platinum drugs like BBR3464 and CT-3610 inside CB[*n*]s have the potential to protect these drugs from degradation, which can lead to an increase in the maximum tolerated dose of the drug and/or an ability to overcome *in vivo* cisplatin resistance.<sup>28</sup>

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Previously we have examined the cytotoxicity and DNA binding of a dinuclear platinum anticancer complex: *trans*-[PtCl(NH<sub>3</sub>)<sub>2</sub>]<sub>2</sub>μ-dpzm]<sup>2+</sup> (di-Pt) where dpzm = 4,4'-dipyrazolylmethane (Fig. 1).<sup>36,37</sup> The metal complex is moderately active in the L1210 and L1210/DDP cell lines<sup>36</sup> and is thus a good model for studying cucurbituril-multinuclear platinum complex interactions for drugs like BBR3464 and CT-3601. Using <sup>1</sup>H and <sup>195</sup>Pt NMR and molecular modelling we have previously studied the physical chemistry of the host-guest complexes of di-Pt with CB[7] and CB[8] and the effect of the CB[*n*]s on the metal complex's rate of DNA binding and on its cytotoxicity.<sup>29,31</sup> In that work we proposed a three-dimensional structure of the host-guest complexes based on molecular modelling.

The development of a new drug into a usable pharmaceutical is dependent not just on *in vivo* safety and efficacy but also on the ability to mass produce it in a consistent form with a known structure and composition. The thermal stability of the compound is also important as unstable compounds can greatly add to their cost of production and significantly affect their shelf-life and safety. Therefore, the ability to synthesise stable and structurally consistent CB[*n*]-platinum drug host-guest complexes is very important.

In this paper we examine the host-guest complex of CB[7] and di-Pt by single crystal and powder X-ray diffraction. The ability of CB[7] to thermally stabilise di-Pt was also examined using thermogravimetric analyses (TGA) and differential scanning calorimetry (DSC). The results of the X-ray structural analysis and DSC/TGA are discussed in the context of drug production and pharmaceutical reformulation.

## Experimental

### Crystal growth

The metal complex di-Pt<sup>36</sup> and CB[7]<sup>38</sup> were dissolved in ~100 mL of water with heating and sonication and left to evaporate slowly over a period of 2–3 weeks to yield colourless, square-shaped crystals.

### Single crystal X-ray structure†

Structure solution and refinement used programs from the SHELX family.<sup>39</sup> Data were measured at station 9.8 of the Daresbury synchrotron radiation source to give; monoclinic, space group *P2*/*c*, *a* = 28.225(8), *b* = 22.859(7), *c* = 29.728(9) Å, β = 98.209(4)°, *V* = 18984(10) Å<sup>3</sup>, *Z* = 10, λ = 0.69460 Å, μ = 3.666 mm<sup>-1</sup>, *T* = 120 K; 139661 reflections, 33459 unique, *R*<sub>int</sub> 0.0665; final refinement to convergence on *F*<sup>2</sup> and with 2651 refined parameters gave *R* = 0.0651 (*F*<sup>2</sup>, 24264 obs. data only) and *R*<sub>w</sub> = 0.2001 (*F*<sup>2</sup>, all data), GOF = 1.108. Data were treated with the SQUEEZE option in PLATON to remove a total of 51 residual electrons (presumed to be partial water) from a total 812 Å<sup>3</sup> of void space.<sup>40</sup>

### X-Ray powder diffraction

A sample was lightly ground in an agate mortar and pestle and filled into a 0.7 mm borosilicate glass capillary. The sample was mounted and aligned on a Bruker-AXS D8 Advance diffractometer (Table 1) and data were collected at room temperature in the

**Table 1** Single crystal X-ray diffraction data for the host-guest complex of CB[7] and di-Pt

| Crystal data                            | Value   |
|---|---|
| Empirical formula                       | C <sub>49</sub> H <sub>71.06</sub> Cl <sub>4</sub> N <sub>36</sub> O <sub>19.53</sub> Pt <sub>2</sub> |
| Formula weight                          | 2010.96 g mol <sup>-1</sup>   |
| Crystal system                          | Monoclinic  |
| Space group                             | <i>P2</i> / <i>c</i>  |
| Unit cell dimensions                    |   |
| <i>a</i> /Å                             | 28.225(8)   |
| <i>b</i> /Å                             | 22.859(7)   |
| <i>c</i> /Å                             | 29.728(9)   |
| α/°                                     | 90.00   |
| β/°                                     | 98.209(4)   |
| γ/°                                     | 90.00   |
| Volume/Å <sup>3</sup>                   | 18984(10)   |
| Density (calculated)/Mg m <sup>-3</sup> | 1.757   |
| Crystal size/mm                         | 0.04 × 0.04 × 0.02  |

range 3–40° 2θ (2 kW; Cu Kα<sub>1</sub>, λ = 1.54056 Å; step size 0.017° 2θ, step time = 2 s). The data from the polycrystalline sample were compared to the single-crystal structure *via* a Pawley<sup>41</sup> refinement as implemented in TOPAS Academic v4.1.<sup>42</sup>

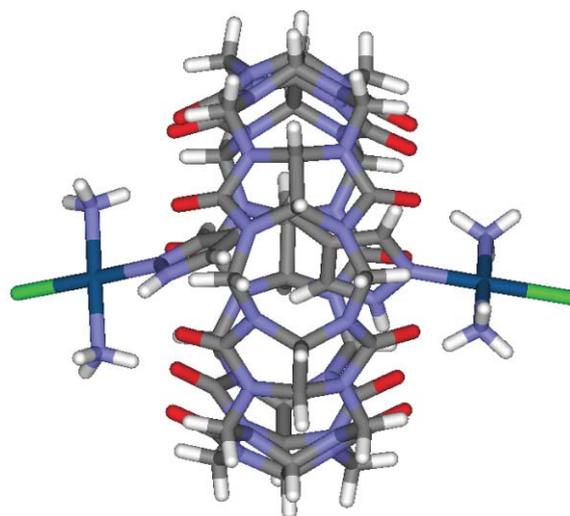
### Thermogravimetric analysis and differential scanning calorimetry

Experiments were conducted using a Mettler Toledo TGA/SDTA 851e and a Mettler Toledo DSC 8222e, respectively. Each sample (approximately 5–10 mg) was placed in an alumina or sealed aluminium pan, and weighed. Samples were then heated at a rate of 10 °C min<sup>-1</sup>. Experiments were performed on multiple, individual batches to confirm product consistency.

## Results and discussion

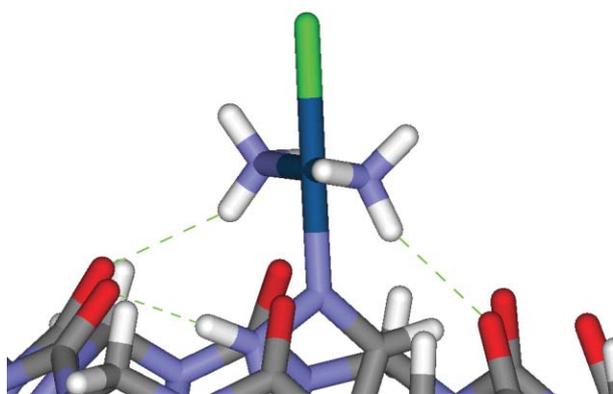
### Single crystal X-ray structure

The single crystal X-ray structure shows that CB[7] and di-Pt form a 1:1 host-guest complex where the metal complex is partially encapsulated within the macrocycle (Table 1 and Fig. 2). The dpzm ligand is located completely within the CB[7] cavity where



**Fig. 2** The single crystal X-ray diffraction structure of the host-guest complex of CB[7] and di-Pt.

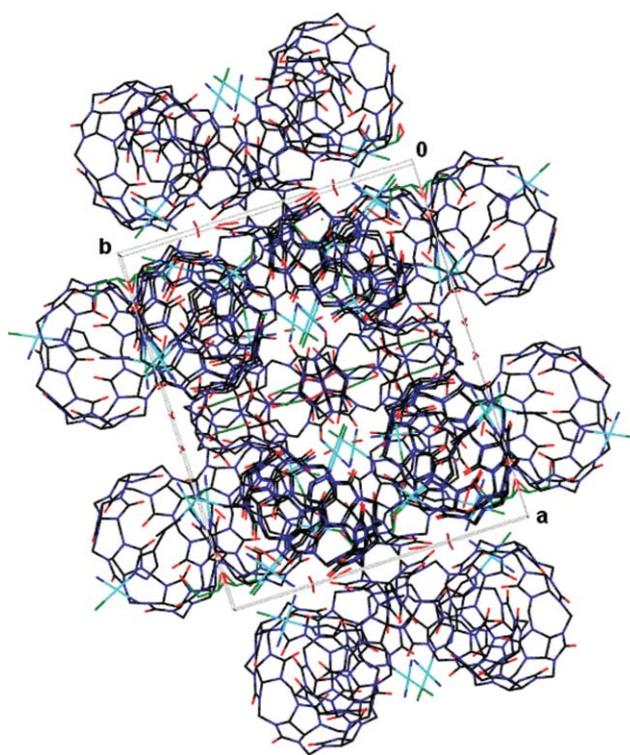
binding is stabilised by hydrophobic forces and two hydrogen bonds (1.98 Å) between both pyrazole amine groups to one CB[7] carbonyl oxygen at each portal. The platinum ammine ligands are located just beyond the CB[7] portals and help to further stabilise the host–guest complex through an additional four hydrogen bonds (2.4 Å) to the CB[7]-carbonyls (Fig. 3). As well as the two chloride counterions of di-Pt, each host–guest complex crystallises with 5.5 water molecules. Whilst the waters are bordering on disordered within the structure some form hydrogen bonds with the carbonyl portals of CB[7] and the ammine groups of di-Pt. The unit cell consists of four asymmetric units, each of which contains three crystallographically independent CB[7]–di-Pt moieties and the associated counterions and waters of hydration (Fig. 4). This is consistent with the X-ray structures of other CB[7] complexes.<sup>43</sup>



**Fig. 3** Hydrogen bonding between di-Pt and CB[7]. Six such hydrogen bonds stabilise the host–guest complex; three at each CB[7] portal.

The single crystal X-ray diffraction structure is in good agreement with our previously proposed molecular model<sup>31</sup> and validates the modelling technique for the study of further drugs with CB[*n*]s where suitable single crystals for analysis cannot be obtained. In the molecular model the Pt–Pt distance was calculated to be 10.05 Å, which is shorter than the crystal determined length of 10.49 Å. In the model the angle of the methylene bonds to the pyrazoles is 114.9°, but is 113.7° in the crystal, and finally, in the molecular model both platinum atoms maintain a perfect square-planar shape with bond angles of 90 (±0.1)°. In the crystal structure, the platinum atoms form a slightly distorted square-planar shape with the bond angles between 88.4 and 91.4°.

The only large difference between the model and the crystal structure is seen in the structure of the CB[7] molecule. The portals of CB[7] are normally symmetrical with a van der Waals radius of 5.4 Å and this symmetry is observed in the molecular model of CB[7] and di-Pt;<sup>31</sup> however, from the single X-ray crystal structure the binding of di-Pt distorts the CB[7] into an ellipsoid shape, with one long van der Waals axis of ~5.9 Å and one shorter axis of 4.8 Å. This structural change appears to be due to the hydrogen bonding between the platinum ammine ligands and the CB[7] carbonyl groups. The height of the CB[7] in both the model and the X-ray structure remains unchanged at 9.1 Å.

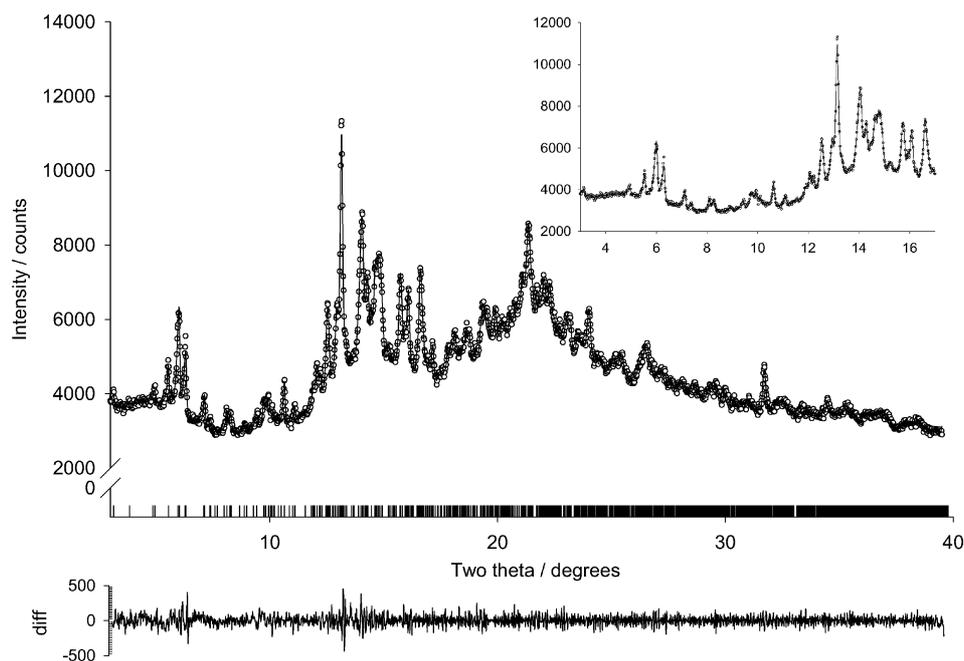


**Fig. 4** The single crystal X-ray diffraction unit cell of the host–guest complex of CB[7] and di-Pt. Each unit cell consists of four asymmetric units made of one CB[7], one di-Pt, two chloride counterions and 5.5 water molecules.

### X-Ray powder diffraction

A Pawley-type fit to the data, in which background, zero-point, peak shape and unit cell parameters describing the profile were refined, yielded an excellent fit to the data with no significant diffraction features unaccounted for ( $R_{wp} = 0.016$ ; refined unit cell parameters  $a = 28.225(8)$  Å,  $b = 22.859(7)$  Å,  $c = 29.728(9)$  Å,  $\beta$  angle = 98.209(4)°; Fig. 5). These values are consistent with slight thermal expansion of the unit cell compared to the single crystal values as a result of the higher temperature of XRPD data collection (XRPD, 273 K; single crystal diffraction data, 120 K). The comparison confirms that the polycrystalline sample is consistent with the single crystal structure.

The production of structurally consistent formulations of host–guest complexes of CB[*n*]s and platinum drugs, in terms of both the host–guest complex formed but also the crystal packing and hydration, is required for new drugs to be developed successfully as pharmaceuticals. Given that single crystal samples may not always be representative of bulk material, the XRPD analysis of CB[7]–di-Pt polycrystalline sample is important to confirm bulk phase identity. This is particularly true when dealing with crystals of CB[*n*]s and their host–guest complexes, as typically all CB[*n*]s crystallize with a large number of water molecules. As discussed earlier CB[7] and di-Pt crystallised with only 5.5 water molecules, but other types of host–guest complexes of CB[7] and platinum complexes, like cisplatin, oxaliplatin and platinum-based DNA intercalators have been shown to contain between 3 and 13 water molecules per CB[*n*]–platinum complex in their structures.<sup>30,43,44</sup> These different hydrate forms can lead to significant changes in crystal packing of



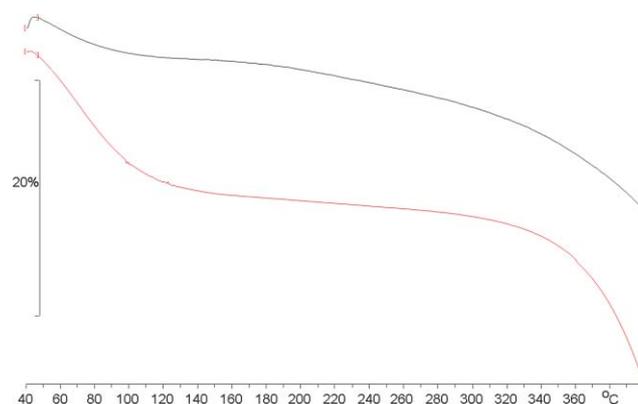
**Fig. 5** Final observed (points), calculated (line) and difference [ $(y_{\text{obs}} - y_{\text{calc}})$ ] profiles for the Pawley refinement to the XRPD pattern of the polycrystalline CB[7]-di-Pt sample. Inset: low-angle region of the pattern highlighting excellent correspondence between observed and calculated profiles.

CB[*n*]s in different samples. Recent studies examining the synthesis of CB[6] in hydrochloric acid using microwave irradiation produced two different CB[6] crystal morphologies. We believe these two morphologies represent CB[6] crystals with differing numbers of water molecules in their unit cells, and thus a different CB[6] w/w composition. When designing a pharmaceutical preparation, it is often critical to separate different crystal types to ensure a consistent amount of drug is delivered. The single polycrystalline phase observed for the bulk crystals of CB[7]-di-Pt is promising for the future application of CB[*n*]s as drug delivery vehicles for multinuclear platinum drugs, as it indicates that these host-guest complexes can be synthesised with high chemical and physical purity.

### Thermal gravimetric analysis

In addition to the problems of consistent synthesis of crystalline forms, the large number of water molecules in CB[*n*] host-guest complexes, including CB[7]-di-Pt, may be problematic in trying to obtain stable pharmaceutical formulations. Loss of one or more water molecules from a CB[*n*]-drug complex could have effects on any component of the formulation, including: a change from one crystalline form to another, a change from amorphous to crystalline material and *vice versa*, or drug degradation. Such changes in the physical form can also affect flowability during automated powder processing, compaction properties of oral formulations, and drug dissolution profile/bioavailability (*e.g.* if administered in a tablet or non-IV dosage form).

Using CB[6] with differing numbers of hydration water molecules, Germain *et al.* examined the dehydration of CB[6] by TGA.<sup>45</sup> Water loss from solids of CB[6] is an endothermic process that occurs between 50 and 200 °C. Similarly, loss of water is seen from the solid powder forms of free CB[7] and the CB[7]-di-Pt complex in their respective TGA spectra (Fig. 6). Water loss begins at 40 °C and is complete in one step by



**Fig. 6** The thermogravimetric trace of (red) free CB[7] and (black) the CB[7]-di-Pt host-guest complex, showing the loss of mass with temperature.

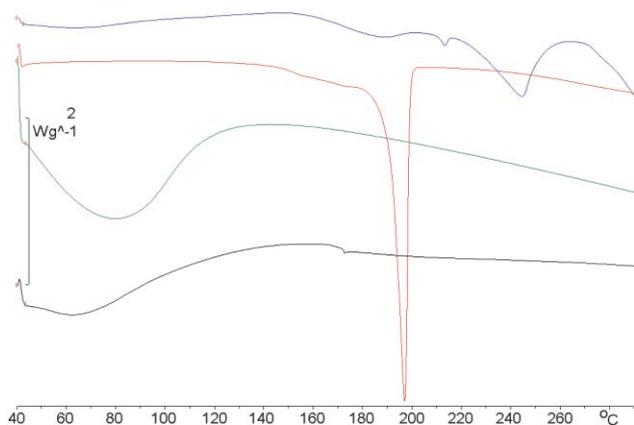
~110 °C. Free CB[7] loses 11% of its mass over this temperature range, corresponding to the dehydration of approximately seven water molecules, which is consistent with the average hydration state of CB[7].<sup>38</sup> The CB[7]-di-Pt complex loses 3.4% of its mass in the same temperature range, which corresponds to the loss of 4 of the 5.5 water molecules. No further loss of water is seen at higher temperatures. An analysis of further individual batches yields similar traces indicating production of material with a consistent hydration state.

These TGA results therefore highlight a problem in developing pharmaceutical preparations of CB[*n*]s with platinum drugs. In order to prevent water loss, a method of drying these host-guest complexes to remove as much water as possible, but without degrading the drug before they are formulated, may need to be developed.

## Differential scanning calorimetry

As well as providing protection to platinum drugs from degradation inside cancer cells, CB[n]s may also act to thermally stabilise drugs from degradation during manufacture and storage. Some platinum drugs, particularly oxaliplatin, are unstable and degrade easily in the solid state or in aqueous solutions.<sup>43</sup> Previously, CB[7] binding has been shown to reduce qualitatively the degradation rate of oxaliplatin, and it was of interest to see if a similar effect acts for multinuclear drugs.<sup>43</sup>

As such, the melting and thermal degradation of free dpzm, di-Pt, CB[7] and the CB[7]–di-Pt was examined by DSC. The spectrum of free dpzm is consistent with other small, unchanged organic molecules (Fig. 7); the compound begins to melt at 186.5 °C and is complete at 198.8 °C. From this spectrum a standard enthalpy of fusion of 27.92 kJ mol<sup>-1</sup> can be calculated for free dpzm. The di-Pt metal complex as a 2+ inorganic salt does not melt at temperatures below 300 °C. As such, the peaks seen in the DSC spectrum of free di-Pt at temperatures around 190 and 245 °C represent two degradation events of the metal complex. Further degradation is seen at temperatures beyond 270 °C. In the DSC spectra of free CB[7] and the CB[7]–di-Pt host–guest complex the only peaks of interest occur between 40 and 130 °C, which represent the loss of water from the system (consistent with the TGA results). No degradation is observed at temperatures up to 290 °C. Overall, the results indicate that CB[7] provides significant thermal stability to di-Pt, which may allow the heating of platinum drugs to temperatures of around 100 °C for prolonged periods to remove the water from the solid formulations of CB[n]s and platinum drugs, thus providing a more stable and reproducible drug product.



**Fig. 7** The differential scanning calorimetry traces of (blue) di-Pt, (red) dpzm, (green) CB[7], and (black) the CB[7]–di-Pt host–guest complex.

## Conclusions

In this paper we examined the host–guest complex of the macrocycle CB[7] with the model dinuclear platinum complex di-Pt. A 1:1 host–guest complex is formed where the platinum complex is partially encapsulated by CB[7] with the dpzm ligand of the metal complex inside the macrocycle cavity and with the platinum groups sitting just beyond the portals. Binding is stabilised by hydrophobic effects and six hydrogen bonds. The bulks crystals of CB[7] and

di-Pt also produce a single polycrystalline phase, possibly making the resultant complex easier to process and formulate, than if different crystal structures (e.g. hydrates) were produced; as is sometimes the case with CB[n]s. Both free CB[7] and the CB[7]–di-Pt host–guest complex contain significant amounts of water within their structures, which may make the synthesis of stable CB[n]–platinum drug formulations difficult. DSC demonstrated that at temperatures up to 290 °C, CB[7] completely protected di-Pt from thermal degradation. This result may indicate that CB[n]–platinum drug complexes may be heated for prolonged time periods in order to remove water from their structures before their formulation into usable dosage forms.

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