Supramolecular Drug Inclusion Complex Constructed from Cucurbit[7]uril and the Hepatitis B Drug Adefovir

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Abstract: The interaction between cucuribit[7]uril (Q[7]) and Adefovir (ADV) has been studied in aqueous solution by ^{1}H NMR spectroscopy, electronic absorption spectroscopy, Isothermal Titration Calorimetry and mass spectrometry. The results revealed that an inclusion complex was formed via encapsulation of the purine rings of the guest ADV, while the phosphonomethoxyethyl group was prevented from entering the cavity. ITC data revealed that the formation of this 1:1 inclusion complex is mainly driven by favourable enthalpy changes. Studies investigating the release of ADV from the inclusion complex revealed enhanced rates under acidic conditions, although the rates were slower than observed for the free guest under the same

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conditions. Thermal stability studies indicated that the included form of ADV was more stable that the free form.

Introduction

Adefovir (ADV) was originally of interest in the 1990s for the treatment for HIV, but complications with dosage size *versus* kidney problems led to its withdrawal by the FDA. [1] However, the use of lower dosages proved fruitful for the treatment of hepatitis B, and in the early 2000s, Adefovir was approved for use. [2] The medicinal potential of compounds such as Adefovir can be broadened and/or improved if delivery to specific targets in the body is achieved without degradation. With this in mind, we are interested in the host-guest properties of cucurbit[*n*]urils, Q[*n*]s, which given their enhanced solubility, recognition properties, ability to cross cell membranes and favorable toxicity profiles are attractive as containers/scaffolds for drug delivery. [3] This is exemplified by the work of Isaacs *et al* who have made use of cucurbit[7]uril to deliver oxaliplatin to cancer cells, [4] whilst Wang *et al* reported reduced toxicity but preservation of anticancer activity for Q[7] encapsulated camptothecin. [5] The host-guest complex formed between Q[7] and oxaliplatin demonstrated enhanced antitumour activity (using colorectal cells) *versus* only oxaliplatin, which illustrated the potential for supramolecular chemotherapy. [6] There is also potential for such an approach to be employed in the war against neurodegenerative diseases, such as Parkinson's disease. [7] Other complexation studies on Q[*n*]s and multinuclear platinum complexes suggested that Q-based

scaffolds can, via encapsulation, provide steric hinderance to drug degradation and thus have potential for pharmacological delivery. [8] Other work by Zheng and Wang has reported how the presence of Q[7] can improve the *in-vitro* and *in-vivo* uptake of the dye molecule coumarin-6; [9] coumarin forms a 1:1 inclusion complex with Q[7] but a 2:1 complex with Q[8]. [10] Other drugs such as atenolol, glibenclamide, memantine and paracetamol can be stabilized in the solid state by forming inclusion complexes with Q[7], [11] whilst increased stability (2 to 3x *versus* similar sized β-cyclodextrin) constants are observed for the anaesthetics procaine, tetracaine, procainamide, dibucaine and prilocaine in aqueous solution. [12] The histamine H_2-receptor antagonist rantidine has also exhibited increased stability in acidic aqueous solution in the presence of Q[7], [13] as did the antituberculosis drugs pyrazinamide and isoniazid. [14] Furthermore, the problematic cardiotoxicity of the antituberculosis drug clofazimine can be almost completely eliminated by complexation with Q[7]. [15] It is against this background that we now report our findings on the interaction of Q[7] with Adefovir (ADV) (see Scheme 1). Results are compared against out earlier study of the pro-virucide Adefovir bis(L-leucine propyl)ester (PMEA-Leu) – see scheme 1, left. [16]

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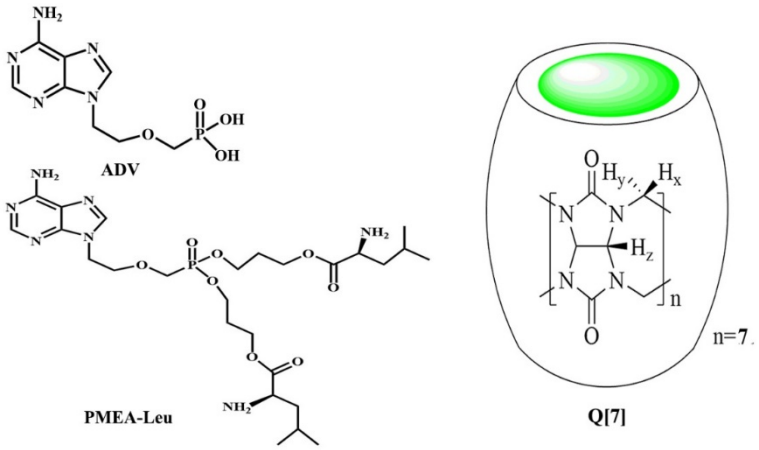

Scheme 1. Schematic molecular structures of ADV, PMEA-Leu and Q[7].

2 Results and Discussion

2.1 *NMR spectroscopy*

In order to investigate the complexation of Q[7] with ADV in solution, ^{1}H NMR spectroscopic titration experiments were first performed by adding increasing amounts of Q[7] into the solution of ADV in D_2O. A slight up-field shift of the signals of the protons of the purine ring was observed when Q[7] was added, and these up-field shifts of the purine ring proton signals can be classified into Ha and Hb. The resonance of the protons Ha, and Hb experience upfield shifts of 0.59 ppm and 0.73 ppm, while the resonances of protons Hc, and Hd associated with the alkyl chain exhibited up-field shifts of 0.55 ppm and 0.39 ppm, respectively. By contrast, the resonance of the proton He experiences a downfield shift of 0.48 ppm when the ratio of ADV/Q[7] reaches 1:1.57. These shift observations indicate that the purine rings were all accommodated within the cavity of Q[7], whereas the phosphonomethoxyethyl was prevented from entering the cavity. These results differ from our early observations for the pro-virucide Adefovir bis(L-leucine propyl)ester (PMEA-Leu), [16] where the two ends of the branches (*ie*. the leucine propyl groups)

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of PMEA-Leu (see scheme 1) were included in the Q[7] cavity. Thus, in the case of the interaction between PMEA-Leu and Q[7], the situation is best described as a partial inclusion complex.

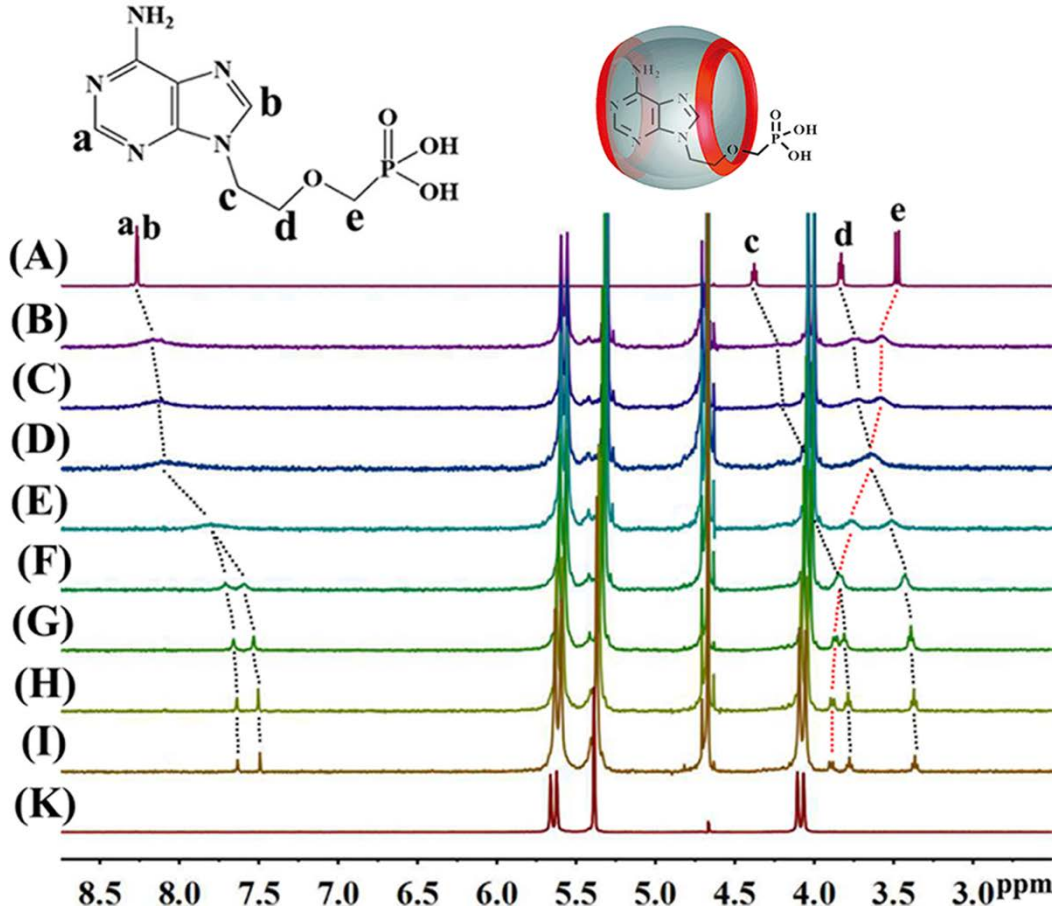

Figure 1. ^{1}H NMR spectra of ADV (A), ADV and Q[7] in the ratio of 0.30 (B), 0.36 (C), 0.47(D), 0.90 (E), 1.0 (F), 1.08 (G), 1.38 (H), 1.57 (I) and Q[7] (K) in D_2O

UV absorption spectroscopy

The supramolecular interactions of the Q[7]/ADV host-guest inclusion complex were then further investigated by the use of UV spectroscopy. As shown in Figure 2A and B, the drug ADV exhibited a maximum UV absorption at 261 nm in aqueous media, whilst Q[7] exhibited no absorbance in the range ≥210 nm. On addition of increasing amounts of the host Q[7] to ADV at a fixed ADV concentration of 4.0×10^{-5} M, the

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absorption spectra of the ADV became weaker. These observations indicate that the interaction between Q[7] and ADV has occurred. Furthermore, in Figure 2C the stoichiometry was confirmed by a Job's plot, and the UV data can be fitted to a 1:1 binding model.

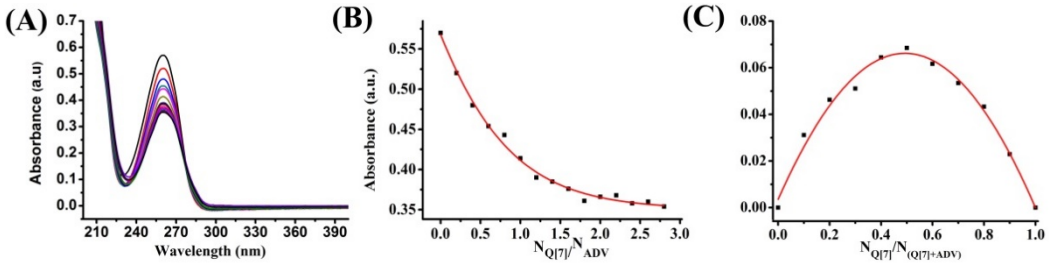

Figure 2. UV absorption of ADV (4.0×10^{-5}mol·L^{-1}) upon the addition of increasing amounts (0, 0.1, 0.2...2.6, 2.8 equiv) of Q[7] (A), the concentrations and the corresponding ΔA *vs.* $N_{iQ[7]}/N_{ADV}$ curve (B) and the $\Delta A \sim N_{Q[7]}/N_{Q[7]+}N_{ADV}$ curves (C).

Isothermal Titration Calorimetry

To study the thermodynamics parameter of the complexation between ADV and Q[7], we conducted ITC experiments at 298.15 K in pure water. The titration graphs and the thermodynamic parameters data are shown in Figure 3 and Table 1 respectively, and the experimental results revealed a K_a value of $(4.25\pm0.22)\times10^4$ M^{-1}. This K_a value is indicative of effective binding between ADV and Q[7]. Furthermore, the negative enthalpy variation, $\Delta H°$= (-29.05±0.13) kJ·mol^{-1} and the negative entropy variation, $T\Delta S°$ = (-2.41±0.26) kJ·mol^{-1}, indicate that the formation of the inclusion complex between ADV and Q[7] is mainly driven by favourable enthalpy changes, accompanied by small negative (unfavourable) entropy changes.

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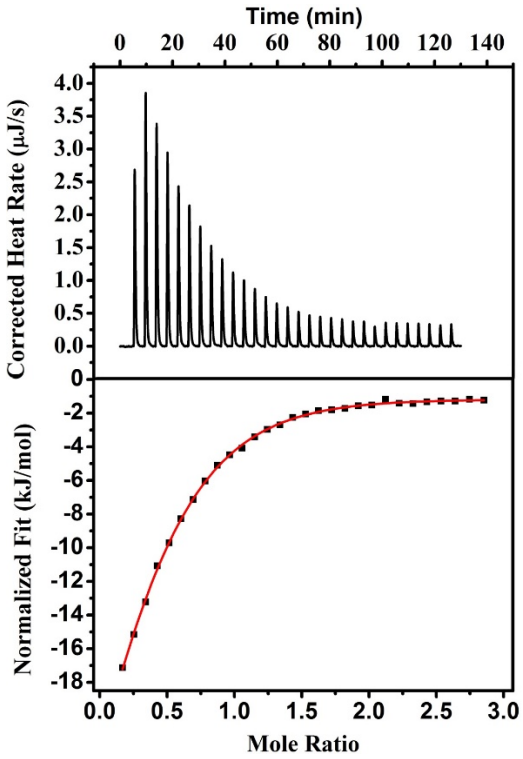

Figure 3 Isothermal titration calorimetry profiles of Q[7] with ADV in aqueous solution at 298.15 K. Nano ITC data for 30 sequential injections of ADV solution into TMeQ[7] solution (top). Apparent reaction heat obtained from integration of the calorimetric traces (bottom).

Table 1. The thermodynamic parameters of ADV/Q[7].

Complex	*n*	K_a (M^{-1})	$\Delta H°$ (kJ mol^{-1})	$T\Delta S°$ (kJ mol^{-1})
ADV/Q[7]	1.07	$(4.25\pm0.22)\times10^4$	(-29.05±0.13)	(-2.41±0.26)

MALDI-TOF mass spectrometry

Analysis of the inclusion complex by MALDI-TOF mass spectrometry revealed (see Figure 4) an intense signal at m/z=1436.01, which corresponds to ADV/Q[7] (calculated 1435.53), thereby providing direct support for the formation of the 1:1 stoichiometry for the host-guest inclusion complex ADV/Q[7].

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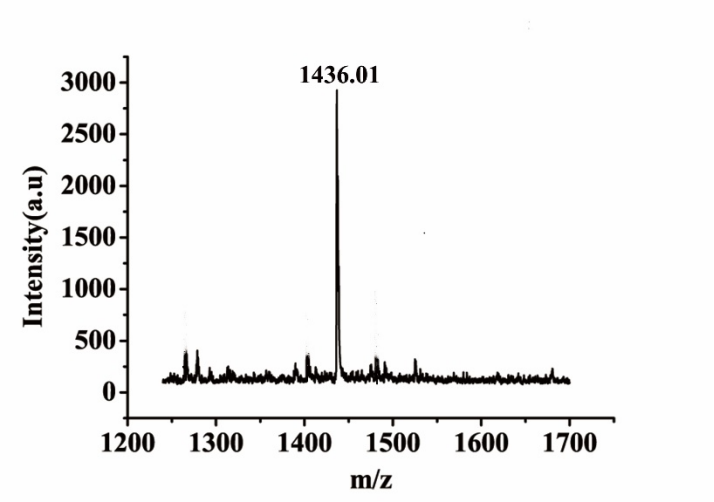

Figure 4. MALDI-TOF mass spectrum of the complex ADV/Q[7].

Controlled release behaviour

To understand the controlled release performance of this inclusion complex, the ADV release from inclusion complex ADV/Q[7] was investigated in water at 37 ℃ (Figure 5). The ADV and ADV/Q[7] are released, via the use of a dialysis bag (for full details see experimental section), on an orbital oscillator and the solution curve for drug release is obtained based on the solution absorption of the drug at different times (Figure 5). When the solution pH was kept at 6.8 using a NaH_2PO_4/ Na_2HPO_4 buffer, the ADV was totally released after 25 min. Whereas, in the case of the inclusion complex ADV/Q[7], the ADV was released from the Q[7] over 145 min, which indicated that the release time of ADV from the inclusion complex was longer than that in the case of the free guest. The released amounts of ADV and ADV/Q[7] were respectively 57.8% and 58.2%. When the pH was about 1.2, the ADV was totally released after 35 min., whilst ADV was released from Q[7] showed over 85 min.; the released amounts of ADV and ADV/Q[7] were 48.7% and 45.1% respectively (Figure 6). In short, these results indicate that the pH of the solvent medium can act as a trigger for release, *ie* for the free drug, the released amounts

of ADV at pH 6.8 was more than at pH 1.2, and similarly for the ADV/Q[7] complex, at pH 6.8, both the release time and the amounts of drug was more than at pH 1.2.

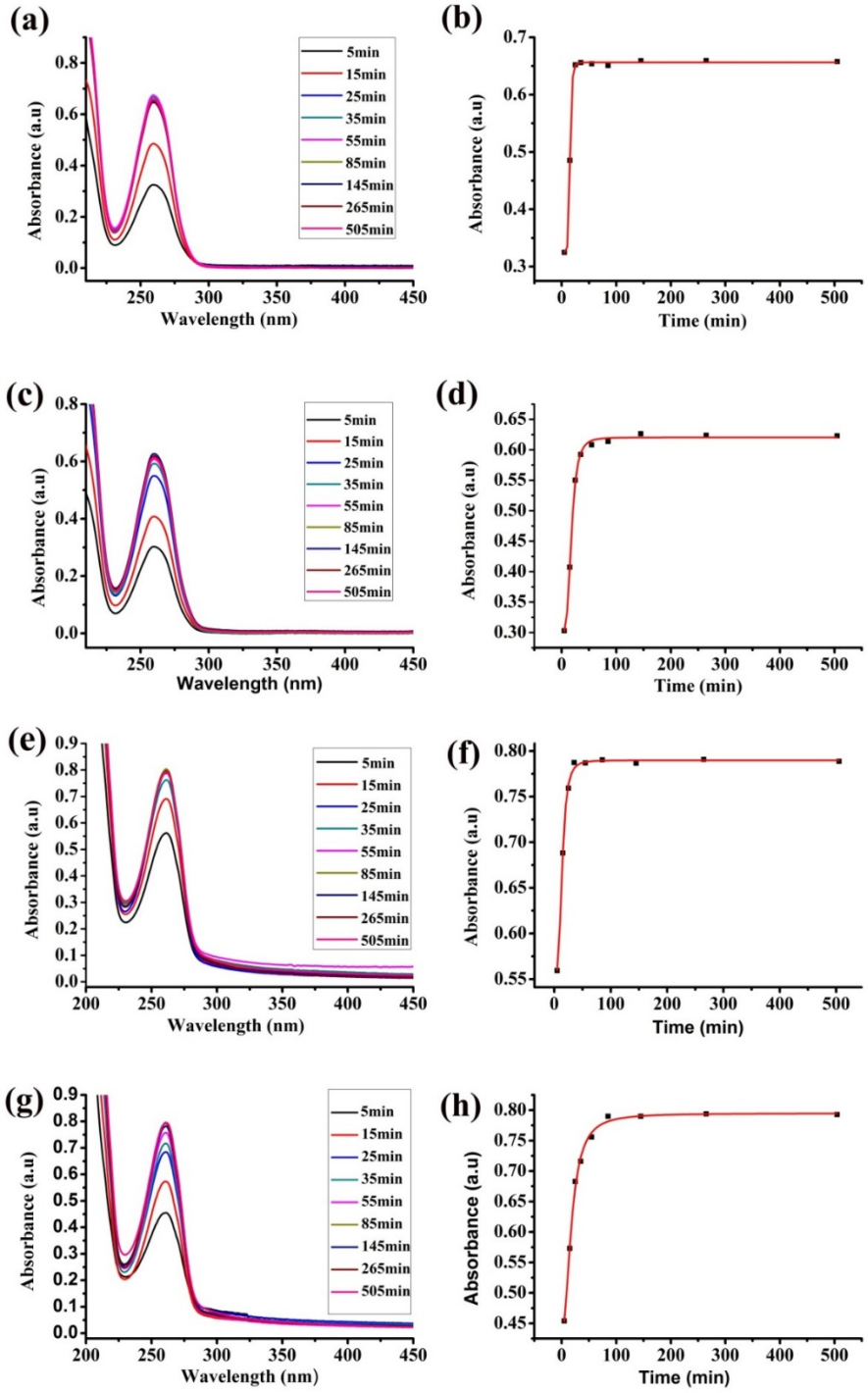

Figure 5. The UV absorption changes in the release for free guest ADV at pH = 6.8 (a, b), inclusion complex ADV/Q[7] at pH = 6.8 (c, d), free guest ADV at pH = 1.2 (e, f) and inclusion complex ADV/Q[7] at pH = 1.2 (g, h) in water.

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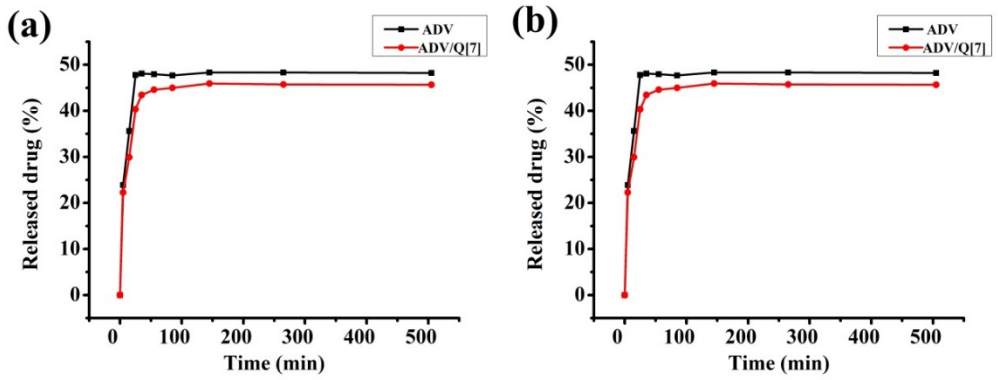

Figure 6. The release curves obtained for free guest ADV and inclusion complex ADV/Q[7] at pH = 6.8 (a), and pH = 1.2 (b) in water.

Thermal stability analysis

From the DTA spectra (Figure 6), it is also evident that ADV and Q[7] interacted with each other. Analysis of the thermal stabilities is via the use of differential scanning calorimetry (DSC) and thermogravimetry (TG). As shown in Figure 7, Q[7] has a broad endothermic peak at 402.1°C typical of an amorphous material, and crystalline ADV has a sharp melting endothermic peak at 306.1°C. Whilst for the ADV/Q[7] inclusion complex a clear melting point endothermic peak is observed at 395.1°C. The results are consistent with the formation of a new material with improved thermal stability of ADV.

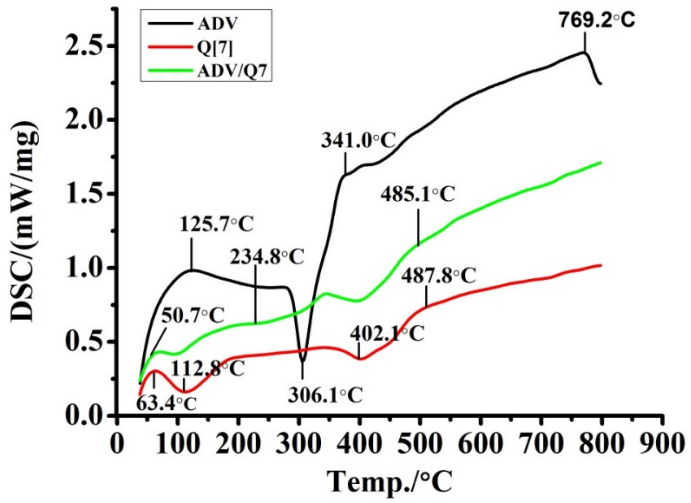

Figure 7. DSC spectra of ADV, Q[7] and inclusion complex ADV/Q[7].

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Conclusion

We have investigated the interaction between cucuribit[7]uril (Q[7]) and the hepatitis B drug Adefovir by a variety of techniques such as ^{1}H NMR spectroscopy, electronic absorption spectroscopy, Isothermal Titration Calorimetry and mass spectrometry. At an ADV:Q[7] ratio in excess of 1:1, NMR observations indicate that the guest purine rings are encapsulated by the Q[7], whilst the phosphonomethoxyethyl was prevented from entering the cavity. ITC results indicate that the formation of the 1:1 inclusion complex is mainly driven by favourable enthalpy changes. The controlled release of ADV from this inclusion complex was investigated in water at 37 ^{o}C at pHs 6.8 and 1.2 using an orbital oscillator. The release was faster under more acidic conditions, though in both cases the process was slower than that observed for the free guest. Analysis of the starting materials and the inclusion complex by differential scanning calorimetry (DSC) indicated that the new inclusion compound afforded a form of ADV that was more thermally stable than the free guest. These studies suggest there is potential to use Q[7] to enhance the stability of ADV-type molecules and to control the release of such drug molecules by manipulation of the pH employed. Further studies are on-going in our laboratory to investigate the ability of Q[7] to act as a scaffold for other drug molecules.

Experimental

Materials and apparatus

The host Q[7] was prepared according to the literature method. [16] Adefovir (ADV) was obtained from Aldrich and was used without further purification. All other

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reagents were of analytical grade and were used as received. Double-distilled water was used for all experiments.

Preparation of ADV/Q[7]: The required amounts of Q[7] and ADV were respectively weighed according to the ratio of $N_{Q[7]}$:N_{ADV} = 1:1, and then dissolved in distilled water and stirred for 30 min. The solvent was then evaporated to afford the 1:1 inclusion compound ADV/Q[7].

^{1}H NMR spectroscopy

To study the host-guest complexation of Q[7] and ADV, all the ^{1}H NMR spectra, including those for the titration experiments, were recorded at 298.15 K on a JEOL JNM-ECZ400S 400 MHz NMR spectrometer (JEOL) in D_2O. D_2O was used as a field-frequency lock, and the observed chemical shifts are reported in parts per million (ppm). The concentration of Q[7] employed in the NMR experiments was1.0 × 10^{-4} mol/L.

UV–vis Absorption measurements

UV-vis absorption spectra of the host-guest complexes were recorded using an Agilent 8453 spectrophotometer at room temperature. The aqueous solution of ADV was prepared with a concentration of 1.00 × 10^{-3} mol/L. An aqueous solution of Q[7] was prepared with a concentration of 1.00 × 10^{-3} mol/L for absorption spectra determination. The UV-vis absorption experiments were performed as follows: 400 μL of a 1.00 × 10^{-3} mol/L stock solution of adefovir and various amounts of an aqueous 1.0 × 10^{-3}mol/L Q[7] solution were transferred into a 10 mL volumetric flask, and then the volumetric flask was filled to the final volume with distilled water. The pH was adjusted to pH =7 with sodium phosphate. Samples of these solutions were combined to give solutions with an ADV:Q[7] ratio of 0, 0.1, 0.2, 0.3, 0.4, 0.5..... and 2.8. The formation constants of the ADV@Q[7] complexes (K) (1:1) were calculated according to curve fitting method. The Jobs plot method was used to determine the inclusion ratio of the substance, $N_{Q[8]}$:$N_{(Q[7]+ADV)}$ = 0, 0.1, 0.2, 0.3, ..., 1.0.

Isothermal titration calorimetry (ITC) experiments

Microcalorimetric experiments were conducted using an isothermal titration

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calorimeter Nano ITC 2G (TA, USA). The amount of heat released was recorded at 298.15 K. The solution of Q[7] (1.00×10^{-4} mol/L) was added to a small amount of thermal reaction cell (1.3 mL) and the free solution (1.00×10^{-3} mol/L) was continuously injected into the syringe (250μL). The dilution heat-corrected reaction of the guest solution was determined in a separate experiment. All solutions were degassed by sonication prior to the titration experiments. Computer simulations (curve fitting) were performed by using the Nano ITC analysis software.

In vitro release studies

The *in vitro* drug release behavior of the inclusion compound was investigated on an orbital oscillator. The 2.7 mg ADV and 14.3 mg ADV/Q[7] (N_{ADV}:$N_{Q[7]}$=1：1) inclusion compound were weighed accurately and then placed on a dialysis bag respectively, and next the sample bags were placed in an orbital oscillator containing artificial intestinal fluid (pH=6.8 phosphate buffer solution) or artificial gastric juice (pH=1.2) hydrochloric acid solution). To this was added an equivalent of hydrochloric acid containing 0.09% Na^+ ion, which was shaken slowly in a water bath at 37°C. At regular time intervals, 1mL of the sample was removed, whilst at the same time adding the same volume of the new buffer solution. The absorbance of the sample was measured at 260 nm.

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