The preparation, characterisation and solubility characteristics of a hydrogen-bonded complex between acyclovir and cytosine

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Abstract

Acyclovir and cytosine form a Crick–Watson hydrogen-bonded complex in dimethylsulphoxide (DMSO). For the complex formation

Acyclovir + cytosine ⇌ complex

the equilibrium constant, K, was determined using NMR spectroscopy to be $K = 1.00 \pm 0.07$ mol^{-1} dm^3 at 21°C in DMSO.

The acyclovir–cytosine complex was formed in DMSO, then diluted with octan-1-ol to leave a 95.3% v/v octan-1-ol/47% v/v DMSO solution, and demonstrated a 12- fold increase in the saturated solubility of acyclovir. This was compared to a solution of acyclovir alone treated in the same manner. This suggests that a complex species of acyclovir with cytosine has a greater lipophilic character than acyclovir alone.

Attempts to increase the octan-1-ol/water partition coefficient for acyclovir produced no significant increase with the presence of cytosine. It was argued that no complexation would occur in water due to the rapid exchange of the protons that are involved in the hydrogen-bonded complex.

Experiments to isolate the solid acyclovir–cytosine Crick–Watson hydrogen-bonded complex were performed. Spectra and T_1 relaxation times obtained during subsequent solid-state ^{13}C NMR experiments provided evidence that a solid complex can be isolated.

Keywords: Acyclovir; Crick–Watson hydrogen-bonded complex; NMR spectroscopy

1. Introduction

Acyclovir, 9-(2-hydroxyethoxymethl) guanine, is a synthetic purine nucleoside analogue that demonstrates strong and selective activity against herpes simplex and selective varicella zoster virus [1]. After being absorbed into the infected cell, acyclovir is first metabolised into acycloguanosine monophosphate. This is further metabolised into the triphosphate which inhibits the virus-specific DNA polymerase.
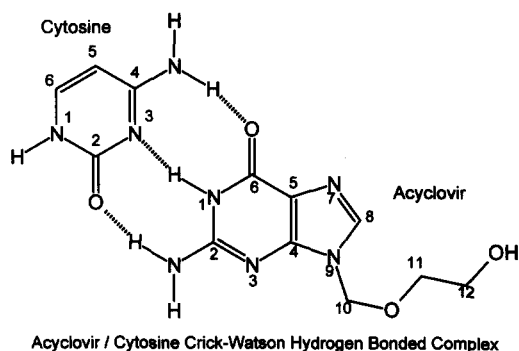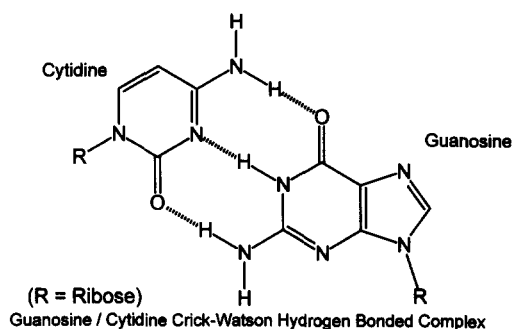
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Fig. 1. Crick–Watson hydrogen-bonded complexes of guanosine–cytidine and acyclovir–cytosine.

2. Experimental

2.1. Chemicals and reagents

Acyclovir and cytosine were obtained from Sigma (Poole, UK). Methanol (HPLC grade), dimethylsulphoxide (AnalaR), octan-1-ol (AnalaR), sodium-dihydrogen orthophosphate (AnalaR) and phosphoric acid (GPR) were obtained from BDH (Poole, UK). Dimethylsulphoxide-d_6 was purchased from Euriso-top (Gif-sur-Yvette, France).

2.2. Partition experiment

Acyclovir (0.1015 g, 4.51×10^{-4} mol) was dissolved in octan-1-ol saturated with water (saturated water) (200 ml). Cytosine (0.0497 g, 4.47×10^{-4} mol) was dissolved in saturated water (200 ml). 2 ml aliquots of the acyclovir solution were then pipetted into five 50 ml volumetric flasks. 2 ml, 4 ml, 10 ml and 20 ml aliquots of the cytosine solution were then added to four of the acyclovir-containing flasks in order to produce solutions with approximately 1:1, 1:2, 1:5 and 1:10 molar ratios of acyclovir to cytosine. Each flask was diluted to volume with saturated water to produce aqueous solutions for examination. The fifth flask was diluted to volume with saturated water to leave a cytosine-free solution. 3 ml aliquots of the five prepared solutions were added to 30 ml of saturated water. The solutions were then shaken mechanically for 6 h, left standing to equilibrate for 24 h and centrifuged to separate the two layers. Both the octan-1-ol layer and the aqueous layer were then assayed for acyclovir by HPLC.

2.3. Octan-1-ol/DMSO solubility experiment

Acyclovir (201.2 mg, 8.94×10^{-4} mol) was dissolved in DMSO (20 ml). Quantities of cytosine (as specified in Table 2) were weighed into vials and 1 ml of the acyclovir solution was added to each vial. A vial was prepared without the addition of cytosine. The cytosine was dissolved by gentle heating. Octan-1-ol (20 ml) was added to the solutions and they were then shaken vigorously for 1 min. The solutions were left at room temperature

Guanosine and cytidine form a Crick–Watson [2] hydrogen-bonded complex in dimethyl-sulphoxide (DMSO) and chloroform [3] (Fig. 1). Direct observation of this complex in water has not been achieved [3]. The structural relationship between guanosine and acyclovir suggests that similar hydrogen-bonded complexation should occur between acyclovir and cytosine (Fig. 1).

In this study the formation of a hydrogen-bonded complex between acyclovir and cytosine was investigated. Following hydrogen bonding, the complexed species is thought to have a greater lipophilic character than acyclovir alone due to the masking of the polar moieties involved in bonding. The lipophilic character of the complexed species was assessed using octan-1-ol solubility experiments. The structure of the complex was assessed using NMR spectroscopy.

(21°C) for 24 h. Precipitate was removed from the resulting suspensions by filtering through a 45 μm syringe filter and the filtrates were assayed directly for acyclovir by HPLC.

2.4. HPLC

A Hewlett-Packard HP 1090 HPLC system consisting of a high pressure pump, variable loop injection system and diode array UV detector was used. A mobile phase of sodium dihydrogen orthophosphate (pH 2.5; 0.02 M) – methanol (92 : 8, v/v), was delivered to 250 mm × 4.6 mm i.d. stainless-steel columns packed with Spherisorb ODS-2 5μ at a flow rate of 1.0 ml min^{-1}. After the elution of acyclovir (≈ 5 min), 100% methanol was delivered to wash any residual octan-1-ol from the column. The column temperature was maintained at 35°C and detection was facilitated using UV at 252 nm. Solutions obtained from the partition and octan-1-ol/DMSO solubility experiments were assayed by injection of 20 ml aliquots and comparison with an external acyclovir standard.

2.5. Determination of complex structure using NMR spectroscopy

Acyclovir (10 mg, accurately weighed) was dissolved in DMSO-d_6 (1 ml) with the gentle addition of heat if required. The solution was filtered through cotton wool into a suitable NMR sample tube and ^{1}H and ^{13}C NMR spectra were recorded on a Jeol 270 MHz FT-NMR spectrometer. The temperature of the NMR tubes during sample acquisition was noted.

The procedure was repeated several times with the addition of a quantity of cytosine (between 3.0 and 18.1 mg) to the acyclovir–DMSO-d_6 solution. ^{1}H and ^{13}C NMR spectra were recorded in the same way. Particular attention was paid to observe the hydrogen-bonded NH protons downfield from 10 ppm in the ^{1}H NMR spectra.

2.6. Preparation and characterisation of solid acyclovir–cytosine complex using ^{13}C solid-state NMR spectroscopy

Complex 1. Acyclovir (0.601 g, 2.38 × 10^{-3} mol) and cytosine (0.301 g, 2.71 × 10^{-3} mol) were dissolved in DMSO (20 ml) and the solution was rotary-evaporated to dryness at 70°C to leave a white granular powder (0.889 g, 98.6% recovery).

Complex 2. Acyclovir (0.604 g, 2.40 × 10^{-3} mol) and cytosine (0.300 g, 2.70 × 10^{-3} mol) were dissolved in DMSO (20 ml) and left to evaporate at 40°C in a flat open dish for 7 days. A white granular powder was produced (0.890 g, 98.4% recovery).

Complex 3. Acyclovir (1.504 g, 6.68 × 10^{-3} mol) and cytosine (0.773 g, 6.96 × 10^{-3} mol) were dissolved in DMSO (50 ml) and the solution was rotary-evaporated at 70°C until the contents were greatly reduced. All solids remained in solution. The flask content weight was 6.361 g. The solution was then placed in a vacuum oven at 40°C for 24 h until a white granular powder was observed (2.206 g, 96.9% recovery).

Acyclovir recovered from DMSO. Acyclovir (0.295 g) was dissolved in DMSO (10 ml) and rotary-evaporated to dryness at 70°C.

Cytosine recovered from DMSO. Cytosine (0.155 g) was dissolved in DMSO (10 ml) and rotary-evaporated to dryness at 70°C.

All samples were run without modification except for grinding. A Varian UNITY plus spectrometer with a 5 mm Doty scientific probe, operating at 75.4 MHz, was used for the experiments. All spectra were recorded at ambient temperature ($\approx 25°C$) and were referenced to an external standard of tetramethylsilane (TMS). Cross-polarisation magic-angle spinning (CP-MAS) experiments were used to obtain ^{13}C spectra. Two indirect ^{1}H relaxation time (T_1) measurements (through the ^{13}C spectrum were carried out on a physical mixture and complex 3.

3. Results and discussion

3.1. HPLC method validation

A linear detector response for solutions of acyclovir between 531 ppm and 53 ppb for a 20 μl injection was demonstrated (correlation coefficient > 0.999). The chromatography was specific for acyclovir–cytosine mixture.

Table 1
Acyclovir–cytosine partition experiment

Sample	[Water] (ppm)	[Octan-1-ol] (ppm)	Partition coefficient
Acyclovir only	16.190	0.329	0.02032
1:1 molar ratio	16.190	0.339	0.02069
1:2 molar ratio	16.274	0.338	0.02077
1:5 molar ratio	16.263	0.338	0.02078
1:10 molar ratio	16.221	0.344	0.02121

3.2. Partition experiment

Traditionally a solvent/water partition coefficient is determined in an attempt to evaluate the lipophilicity of a drug; octan-1-ol is the solvent most commonly used. The higher the partition coefficient, the greater the amount of drug transferred from the aqueous phase into the octan-1-ol and the greater the lipophilicity of the drug. Attempts to increase the partition coefficient of acyclovir by addition of cytosine yielded the results given in Table 1.

Only a very small increase in the partition coefficient can be observed. However, the difference is within analytical error of the HPLC injector reproducibility. The analogues guanosine and cytidine are reported not to complex in D_2O [3]. Rapid exchange of the hydrogen-bonded protons of guanosine and cytidine in water would be expected, making a coordinated hydrogen-bonded complex unlikely. Similarly, acyclovir and cytosine would be expected to follow this behaviour pattern, explaining why no significant increase in partitioning was observed. In the pH 2.5 buffer of the HPLC mobile phase, the additional exchangeable protons should disrupt any complex introduced, allowing the acyclovir to be assayed.

3.3. Octan-1-ol/DMSO solubility experiment

The partition coefficient experiments led to the belief that transfer of an acyclovir–cytosine complex from water to octan-1-ol was not feasible. Acyclovir is however practically insoluble in most orgainic materials [4]. The hydrogen-bonded complex for the analogues guanosine and cytidine was determined by NMR experiments using DMSO as the solvent [5].

DMSO is a solvent possessing a high dielectric constant resulting in a high solvating power. The high degree of polarity in the molecule would be expected to disrupt hydrogen bonding but not to the same degree as water because DMSO contains no hydrogen-bonded protons. Acyclovir is reasonably soluble in DMSO. By adding a large quantity of octan-1-ol to a solution of acyclovir in DMSO, precipitation of acyclovir should occur due to the limited solubility in octan-1-ol already demonstrated. In the presence of cytosine, if complexation occurs the complex should have increased solubility in the mainly octan-1-ol mixture because of the greater suspected lipophilicity of the complex. The concentrations of acyclovir found in the 95.3% octan-1-ol/4.7% DMSO solutions after 24 h at 21°C and the corresponding amount of cytosine added are tabulated in Table 2 and displayed in Fig. 2.

As the concentration of cytosine was increased, the concentration of acyclovir determined in the

Table 2
Concentration of complex in 95.3% Octan-1-ol/4.7% DMSO

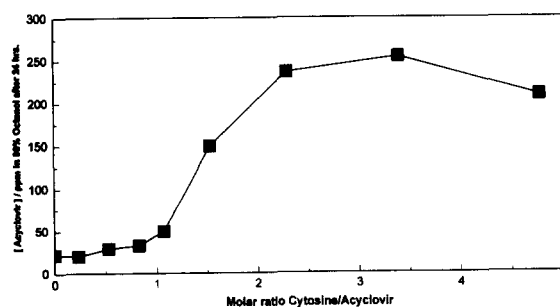| Acyclovir (μmol)	Cytosine (μmol)	Molar ratio	Acyclovir conc.[a] (ppm)
44.66	0.00	0.000:1	22.26
44.66	10.43	0.234:1	20.15
44.66	23.56	0.528:1	29.27
44.66	36.78	0.824:1	32.93
44.66	47.75	1.069:1	49.64
44.66	68.17	1.526:1	149.6
44.66	102.07	2.285:1	236.6
44.66	151.44	3.391:1	254.4
44.66	213.22	4.774:1	209.0

Fig. 2. Acyclovir concentration determined in 95.3% octan-1-ol/DMSO as a function of increasing cytosine concentration.

Table 3
Location of ^{1}H complexation chemical shifts

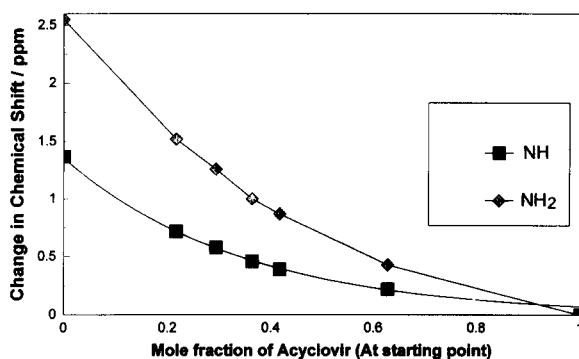| Concentration of acyclovir (mg ml^{-1})	Concentration of cytosine (mg ml^{-1})	^{1}H δ_{NH_2} (ppm)	^{1}H δ_{NH_2} (ppm)
10.2	0	6.50	10.65
10.4	3.05	6.72	11.08
10.2	7.04	6.89	11.52
10.4	8.98	6.96	11.65
10.6	12.62	7.08	11.91
10.1	18.06	7.22	12.17

Fig. 3. Change in acyclovir ^{1}H NMR chemical shifts observed for the NH and NH$_2$ protons as the mole fraction of acyclovir is varied by the addition of cytosine to the DMSO-d_6 solutions.

octan-1-ol solution increased, as demonstrated in Fig. 2. Up to a 12-fold increase in solubility was observed. The baseline value of 22 ppm, where no cytosine was added, compares to a previous determination of 14.4 ppm for the saturated solubility of acyclovir in pure octan-1-ol. This suggests that 4.7% DMSO has a negligible effect on the solubilities in pure octan-1-ol alone, providing good evidence that the complexed species has a greater lipophilic character than acyclovir alone.

3.4. Determination of complex structure using NMR spectroscopy

NMR spectroscopy can be used to study the chemical equilibrium in any reaction in which the chemical shift of a magnetic nucleus in the product differs from the chemical shift of the same nucleus in the reactant [6]. If there is a rapid equilibrium between products and reactants, only

the weighted average chemical shift is observed in the NMR spectrum.

Hydrogen bonding provides an example of this situation as a hydrogen-bonded proton moves downfield over 1.5 ppm from a non-hydrogen-bonded proton [7]. The moving of the hydrogen-bonding protons in the acyclovir–cytosine complex should therefore be evidence of the complex formation being in line with the above theory. If no movement of protons other than the hydrogen-bonded protons within the complex is observed then the sites of interaction can be confirmed.

Furthermore, if it is assumed that the only interactions present are that of a 1:1 complex with no other homonuclear interactions then for such a complexation

Acyclovir + cytosine $\rightleftharpoons$ complex
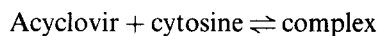
the equilibrium constant

$$K = \frac{x}{(A_0 - x)(C_0 - x)}$$

and the average chemical shift of the acyclovir protons is given by

$$\delta_{obs} = \frac{(A_0 - x)}{A_0} \cdot \delta_{acyclovir} + \frac{x}{A_0} \cdot \delta_{complex}$$

where x is the concentration of complex formed, A_0 is the initial concentration of acyclovir, C_0 is the initial concentration of cytosine, δ_{obs} is the observed shift in the experiment, $\delta_{acyclovir}$ is the shift of acyclovir alone and $\delta_{complex}$ is the shift of theoretical 100% complex [8].

By considering the positions of the NH$_2$ and NH protons observed in the ^{1}H NMR spectra at equilibrium (Table 3), the experimental data was used to solve the two equations in order for values of K and $\delta_{complex}$ to be calculated. The mathematical relationship holds providing a 1:1 complex is present.

First, $\delta_{complex}$ was estimated by plotting the mole fraction of acyclovir, from the total moles of acyclovir and cytosine, against observed proton chemical shift (Fig. 3). As the mole fraction of acyclovir tends to zero, the amount of complex formed from acyclovir will tend to 100%. The estimated values for $\delta_{complex}$, 7.87 ppm for NH$_2$

Table 4
Data from NH_2 proton shifts[a]

Weight acyclovir (mg)	Moles of acyclovir (μmol)	Weight cytosine (mg)	Moles of cytosine (μmol)	Mole fraction acyclovir	Molar ratio	δNH_2 (ppm)	Moles of complex (μmol)	% complex	K (mol^{-1} dm^3)
10.2	45.29	0.00	0.00	1.0000	0.000:1	6.50	0.00	0.0	–
10.4	46.18	3.05	27.43	0.6274	0.594:1	6.72	8.26	17.8	1.1364
10.2	45.29	7.04	63.31	0.4171	1.398:1	6.89	14.36	31.7	0.9485
10.4	46.18	8.98	80.76	0.3638	1.749:1	6.96	17.27	37.4	0.9410
10.6	47.07	12.62	113.49	0.2932	2.411:1	7.08	22.20	47.2	0.9774
10.1	44.85	18.06	162.41	0.2164	3.621:1	7.22	26.25	58.5	1.0369
5.1	22.65	5.47	49.19	0.3152	2.172:1	6.86	6.63	29.3	0.9722
								Mean	1.0021
								%RSD	7.38%

[a] $\delta_{complex}$ estimated from curve (Fig. 4) = 7.87 ppm; $\delta_{complex}$ from iteration = 7.73 ppm.

Table 5
Data from NH proton shifts[a]

Weight acyclovir (mg)	Moles of acyclovir (μmol)	Weight cytosine (mg)	Moles of cytosine (μmol	Mole fraction acyclovir	Molar ratio	δ_{NH} (ppm)	Moles of complex (μmol)	% Complex	K (mol^{-1} dm^3)
10.2	45.29	0.00	0.00	1.0000	0.000:1	10.65	0.00	0.0	–
10.4	46.18	3.05	27.43	0.6274	0.594:1	11.08	7.35	15.9	0.9437
10.2	45.29	7.04	63.31	0.4171	1.398:1	11.52	14.59	32.2	0.9759
10.4	46.18	8.98	80.76	0.3638	1.749:1	11.65	17.10	37.0	0.9242
10.6	47.07	12.62	113.49	0.2932	2.411:1	11.91	21.97	46.7	0.9560
10.1	44.85	18.06	162.41	0.2164	3.621:1	12.17	25.25	56.3	0.9391
5.1	22.65	5.47	49.19	0.3152	2.172:1	11.42	6.46	28.5	0.9336
								Mean	0.9454
								%RSD	1.94%

[a] $\delta_{complex}$ estimated from curve (Fig. 4) = 13.20 ppm; $\delta_{complex}$ from iteration = 13.35 ppm.

and 13.20 ppm for NH, were then used as a starting point to solve the simultaneous equations by an iterative process (Tables 4 and 5).

The data obtained fitted the mathematical model and both the equilibrium constant K and $\delta_{complex}$ have values that hold for each set of chemical shifts obtained. This is consistent with the formation of a 1:1 Crick–Watson [2] hydrogen-bonded dimer. As no movement of other protons was observed, this provides evidence that no other significant interactions occur.

The large movements of chemical shifts seen are typical of those expected from hydrogen bonding. The chemical shift movement for the acyclovir NH proton is exactly twice the shift movement for

the NH_2 proton, as can be seen in Fig. 3. The chemical shift of a hydrogen-bonded proton is independent of whether the proton is in an N–H···N bond or an N–H···O=C bond [8]. This shows that only one of the NH_2 protons is hydrogen-bonded and a dynamic equilibrium between the two protons is set up, giving rise to a single NMR signal. This information is also consistent with the Crick–Watson structure.

The data obtained fitted the model discussed and produced equilibrium constants for the amine peak of $K = 1.00 \pm 0.07$ mol^{-1} dm^3 and for the NH peak of $K = 0.95 \pm 0.02$ mol^{-1} dm^3. Both results are within analytical error of each other. Measurement of the peak locations became

Table 6
^{13}C NMR chemical shifts (δ in ppm)

Acyclovir (mg ml^{-1})	Cytosine (mg ml^{-1})	C=O (6)	C (2)	C (4)	C (5)	C (8)
10.2	0	156.802	153.855	151.412	116.462	137.755
10.4	3.05	157.180	153.945	151.573	116.372	137.827
10.2	7.04	157.467	153.981	151.663	116.246	137.845
10.4	8.98	157.611	154.017	151.735	116.210	137.881
10.6	12.62	157.809	154.053	151.807	116.156	137.889
10.1	18.06	158.042	154.125	151.915	116.120	137.953

difficult as the bands broadened, due to the hydrogen bonding. This was a contributing factor in the observed errors. Assumptions that only a 1:1 complex was formed are justified by the results. Any other interactions, such as homomolecular stacking, if significant, would have affected the results. Clearly these are not dominant factors.

The ^{13}C NMR spectra allowed investigation of the carbon skeleton of the complex after assignment of each signal [6]. In particular the downfield shift observed in the hydrogen-bonded carbonyl of the acyclovir confirmed the third location of the interaction (Table 6). Table 6 shows the movement of the heteroaromatic ring structure during the NMR experiments. Position 6, that of the carbonyl involved in the Crick–Watson hydrogen-bonded structure, can be seen to move downfield as the degree of hydrogen bonding increased. Carbon positions 2, 4 and 8 also move downfield as expected due to mesomeric conjugative effects. Carbon 5 moves upfield, again

Fig. 4. Change in acyclovir ^{13}C NMR chemical shifts for the carbon skeleton observed as the mole fraction of acyclovir is varied by the addition of cytosine to the DMSO-d_6 solutions.

as expected, through mesomeric conjugation (Fig. 4). Fig. 4 shows that the strongest movement in the observed chemical shift is that of the carbonyl carbon involved in a hydrogen bond. With the exception of the C2 carbon, the effect can be seen to decrease with distance away fom the active carbonyl. The C2 carbon is bonded to the amine group which is also involved with hydrogen-bonding interactions. Any other interactions throughout the heteroaromatic ring structure, such as a possible hydrogen bond at N7, N9 or N3, would result in variations in the observed movement of the chemical shift. This provides further evidence for the assumption that no other significant interactions are taking place other than the formation of the 1:1 complex under investigation.

The ^{13}C data were also used to calculate the equilibrium constant following an estimation of $\delta_{complex} = 159.00$ ppm for the C6 carbonyl from Fig. 4. The percentage change of the ^{13}C NMR signal locations, when compared to that of the ^{1}H proton signal movements, is small and extrapolation of the data to estimate $\delta_{complex}$ may involve a large degree of uncertainty. However, this is arbitrary as the estimation is just a convenient starting point for the iterative process to solve the simulateous equations postulated for the data. The data obtained for the C=O peak (Table 7) fitted the model discussed and gave a result of $K = 1.06 \pm 0.07$ mol^{-1} dm^3. This result is within experimental error for the ^{1}H NMR data.

The structure of an observed complex formed in DMSO between acyclovir and cytosine is almost certainly the well-known Crick–Watson structure. An equilibrium constant for the complexation in DMSO at 21°C was calculated to be
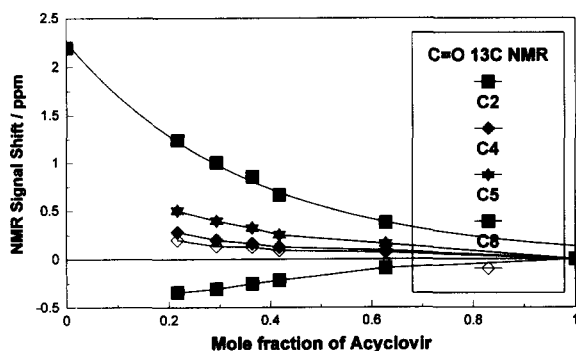
Table 7
Data from C=O carbon shifts[a]

Weight acyclovir (mg)	Moles of acyclovir (μmol)	Weight cytosine (mg)	Moles of cytosine (μmol)	Mole fraction acyclovir	Molar ratio	$\delta_{C\,O}$ (ppm)	Moles of complex (μmol)	% Complex	K (mol^{-1} dm^3)
10.2	45.29	0.00	0.00	1.0000	0.000:1	156.802	0.00	0.0	–
10.4	46.18	3.05	27.43	0.6274	0.594:1	157.180	8.40	18.2	1.1686
10.2	45.29	7.04	63.31	0.4171	1.398:1	157.467	14.49	32.0	0.9641
10.4	46.18	8.98	80.76	0.3638	1.749:1	157.611	17.98	38.9	1.0155
10.6	47.07	12.62	113.49	0.2932	2.411:1	157.809	22.81	48.5	1.0369
10.1	44.85	18.06	162.41	0.2164	3.621:1	158.042	26.76	59.7	1.0909
								Mean	1.0552
								%RSD	6.61%

[a] $\delta_{complex}$ estimated from curve (Fig. 5) = 159.00 ppm; $\delta_{complex}$ from iteration = 158.88 ppm.

1.00 ± 0.07 mol^{-1} dm^3. This was confirmed by consideration of two ^{1}H chemical shift changes and one ^{13}C chemical shift change at the hydrogen-bonding sites.

3.5. Characterisation of solid acyclovir–cytosine complex using ^{13}C solid-state NMR spectroscopy

The ^{13}C spectra of acyclovir and cytosine recovered from DMSO were assigned (Tables 8 and 9 respectively). In the acyclovir spectrum, four of

Table 8
Acyclovir solid-state ^{13}C NMR

Chemical shift, δ (ppm)	Carbon number
58.6 and 62.1	12
72.6	10 + 11
114.9 and 117.2	5
135.6 and 139.6	8
149.7 and 152.3	4
154.7	2
158.8	6

Table 9
Cytosine solid-state ^{13}C NMR

Chemical shift, δ (ppm)	Carbon number
92.6	5
144.7	6
159.6	4
168.2	2

the eight carbons each give rise to two lines of almost equal intensity. Given the slightly broader (compared to others) 158.8 ppm signal it is possible that the signal from carbon 2 is also a doublet with one line being coincident with the signal from carbon 6. A mixture of polymorphs could give a spectrum like this; however, given the nature of the splittings of equal relative intensity and the number of signals showing them it is more likely that they arise from a crystallographic effect. The cytosine spectrum is more straightforward: four carbon nuclei, four signals.

Complex 1 gave a spectrum that indicated a superposition of those of the pure raw materials. This was verified by obtaining a spectrum from a physical mixture of acyclovir and cytosine (equimolar amounts) and using spectral subtraction. Complex 2 gave a spectrum immediately identified as not being a simple superposition of the spectra from the pure materials. The spectrum did contain some signals that could be attributed to pure acyclovir. These were subtracted out to leave a "pure complex" spectrum. Particularly striking in the spectrum is the collapse of the acyclovir C4, C5, C8 and C12 doublets to single lines at the respective average positions. Three lines appear for the cytosine C5 carbon (none of which equate to its position in the pure material). The two high frequency cytosine signals also show some small change in position but one of these, the cytosine C4, is complicated by overlap with the C6 acyclovir carbon. Whether this is a mixture

of acyclovir and cytosine in different crystallographic forms to these of the pure materials recovered from DMSO cannot be proved from the spectra. Complex 3 gave a spectrum similar to that of complex 2. A subtraction (complex 3 − complex 2) showed very little difference.

In the solid state, protons tend to be strongly coupled and a single "average" T_1 relaxation time is observed for the whole molecule. In a physical mixture of two molecules with different T_1 values their relaxation times should behave differently. The ^{1}H T_1 values measured from the ^{13}C spectrum in the physical mixture of acyclovir and cytosine (complex 1) showed a clear difference for acyclovir (10 ± 2 s) and cytosine (16 ± 1 s). Repeating the experiment for complex 3 resulted in all signals having, within experimental error, a common ^{1}H T_1 (9 ± 2 s). This is evidence that acyclovir and cytosine are strongly interacting, i.e. - they form a complex. However, the form of cytosine in the "complex" might have the same T_1 value as acyclovir so this result is probable but not absolute proof.

4. Conclusion

An attempt was made to alter the physicochemical properties of acyclovir by forming a hydrogen-bonded complex of the drug. Formation of a Crick–Watson hydrogen-bonded complex between acyclovir and cytosine has been demonstrated in DMSO and an equilibrium constant for the 1:1 complex formation was determined to be 1.00 ± 0.07 mol^{-1} dm^3 at 21°C. Determination of the solubilities in 95.3% octanol/4.7% DMSO has revealed a significantly greater lipophilicity for the complex with cytosine than that of acyclovir alone.

Evidence was also provided, in the form of solid-state NMR, for the successful preparation of a solid acyclovir–cytosine complex. Further work is required in order to fully characterise this material.

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