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Novel non-acidic formulations of haloperidol complexed with β-cyclodextrin derivatives

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Abstract

Haloperidol (Hal), a highly hydrophobic drug, was complexed with two β-cyclodextrin (β-CD) derivatives. Hal solubility was increased 20-fold in the presence of a 10-fold excess of methyl β-CD (Meβ-CD) and 12-fold in the presence of a 10-fold excess of 2-hydroxypropyl β-CD (HPβ-CD). The stoichiometries and stability constants of Hal–Meβ-CD (1:1 and 2345 M^{-1} at 27°C) and Hal–HPβ-CD (1:1 and 2112 M^{-1} at 27°C) complexes were calculated by the continuous variation and phase solubility methods respectively. Differential scanning calorimetry and ^{1}H-NMR were used to confirm the formation of inclusion complexes. Moreover, the enthalpy and entropy of the complexation process were calculated for both complexes in order to obtain such information as the main 'driving force' and whether or not complex formation is thermodynamically favoured. This was achieved by monitoring the isothermic solubility lines at various temperatures. © 1997 Elsevier Science B.V.

Keywords: Haloperidol; Cyclodextrins; Complexation; Drug solubilization; DSC; NMR; Stability constant; Enthalpy; Entropy

1. Introduction

Haloperidol (Hal) is a butyrophenone neuroleptic used as a central nervous system anti-depressant, notably in the treatment of schizophrenia, mania and similar psychotic states [1]. As Hal is practically insoluble in water (1.4 mg 100 ml^{-1}) [1], pharmacologically active doses of the drug are administered in acidic aqueous media (in which its solubility increases) with a pH range of 2.5–3.8 (injectable form) and 2.5–4.5 (oral form) [1]. However, such acidic solutions can act as irritants and, therefore, new formulations in neutral aqueous solutions are required.

A well known [2] approach for the solubilization of insoluble drugs in neutral pH is drug complexation with cyclodextrins (CD). These are α-1,4 linked cyclic oligosaccharides of D-glucopyranose units, known to form non-covalent water-soluble inclusion complexes with a wide variety of

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drugs. Such complexation increases drug solubility and can also improve drug stability [3] or bioavailability [4]. β-Cyclodextrin (β-CD) is of appropriate size and shape to interact efficiently with numerous drug substances [5] although its relatively low solubility [6] leads to toxic manifestations when used parenterally [7]. CD derivatives, especially methylated or hydroxypropylated, are used extensively as their aqueous solubility is much higher than that of the natural CD and their haemolytic activity and toxicity (e.g. irritation) are reduced [8].

In the present study solubilization of Hal was achieved by complexation with two derivatives of β-CD, namely 2-hydroxypropyl-β-CD (HPβ-CD) and methyl-β-CD (Meβ-CD). The stability constant of the complexes and their stochiometry were estimated by the phase solubility technique using the linear model of Higuchi-Connors and the continous variation plot respectively. Additional information on the complexation process (e.g. entalpy and entropy) was obtained by monitoring the isothermic solubility lines.

2. Experimental procedures

2.1. Materials

Hal was obtained from Sigma (Poole, Dorset, UK); HPβ-CD and Meβ-CD were from Wacker Chemie (Munich, Germany). HPβ-CD has a degree of substitution (DS) of 0.4 (number of hydroxypropyl groups per unit of anhydroglucose) and a relative molecular mass (M_r) of 1300. The DS value (a measure of the extent to which the reactive hydroxyls in each glucose unit of the ring have been substituted) obtained by digital integration, was confirmed from the ^{1}H-NMR spectrum of HPβ-CD in deuterium oxide. Similarly, Meβ-CD has a DS value of 1.8 and a M_r of 1325. Deuterium chloride (DCl) and deuterium oxide (D_2O) were purchased from Fluka (Poole, Dorset, UK); Double distilled water was obtained through a MilliQ system, (Waters). All other reagents were of analytical grade.

2.2. Instrumentation

A Compuspec UV/visible spectrophotometer (Wallac) connected to a personal computer was used throughout the studies. Characterization of the Hal–HPβ-CD and Hal–Meβ-CD complexes in solid state was carried out by differential scanning calorimetry (DSC). Thermograms were obtained in a Perkin Elmer DSC 7 differential scanning calorimeter using vented aluminium pans. Typical conditions were: temperature range, 50–300°C; scanning rate, 10°C min^{-1}; sample weight, 10 mg. Baseline optimization was performed before each run. Characterization of the Hal–CD complexes in aqueous solutions was carried out by ^{1}H-NMR spectroscopy. ^{1}H-NMR spectra were obtained in 10% DCl and recorded on a Brucker AM 500 spectrometer connected to an Aspect 3000 computer. The chemical shifts were related to the residual solvent signal (hydrogen–deuterium chloride = 4.84 ppm at 293 K). Typical conditions were 16 K data points with zero filling, sweep width of 5 kHz giving a digital resolution of 0.61 Hz point^{-1}, pulse width 4 μs, acquisition time 1.64 s and number of scannings 128 for the complexes and 640 for pure Hal due to its low solubility.

2.3. Preparation of complexes

The inclusion complexes of Hal with the β-CD derivatives were prepared by the freeze-drying method [9]: 0.08 mmol HPβ-CD or Meβ-CD was dissolved in 5 ml distilled water and the clear solutions were added dropwise to a suspension of Hal in water (0.08 mmol suspended in 5 ml) under continuous stirring. The mixtures were stirred in the absence of light at room temperature for 4 days and then filtered through a 0.22 μm membrane filter unit (Millex^R-HV, Millipore, Bedford, MA). The filtrates were freeze-dried to yield amorphous powders.

2.4. Solubility studies

Solubility studies were performed according to the method of Higuchi and Connors [10] which monitors the increase in Hal solubility in the

presence of increasing concentrations of CD. The principle of the method is based on the fact that by adding increasing concentrations of CD the equilibrium, expressed as

$$\mathrm{Hal} + \mathrm{CD} \underset{k_d}{\overset{k_r}{\rightleftarrows}} \mathrm{Hal{:}CD} \qquad K_{st} = \frac{k_r}{k_d}$$

(where K_{st} is the stability constant and k_r, k_d are respectively the recombination and the dissociation rate constants of complex formation), is shifted to the right (complex formation). As the complex itself is more soluble than Hal, the overall result is an increase in Hal solubility.

Solubility experiments were performed in 25 ml brown glass vials immersed in a thermostat controlled shaking water bath (SS40—A5 Grant Instruments, Cambridge, UK) at 27, 37, 47 and 57°C. Each vial contained identical amounts of Hal in considerable excess of its normal solubility. Good wettability of Hal with the aqueous phase was attained by sonicating each suspension for up to 5 min at 20°C. In each vial a fixed volume of water containing increasing concentrations of HPβ-CD or Meβ-CD was added. On reaching equilibrium of complex formation (after about 4 days), the suspensions were filtered through 0.22 μm membrane filters and filtrates were freeze-dried. The powders obtained were dissolved in methanol and Hal concentration was calculated using a calibration curve for the drug in methanol.

2.5. Continuous variation plots (job plots)

A reliable determination of complex stoichiometry can be provided by the continuous variation technique (Job plot) [11], based on the difference in absorbance ($\Delta A = A - A_0$) of Hal observed in the presence (A) and absence (A_0) of CD. Thus, equimolar solutions of Hal and the two cyclodextrins (HPβ-CD and Meβ-CD) were prepared and mixed to standard volume and proportions so as to obtain constant total concentration values for the materials in the mixtures ($[\mathrm{Hal}]_t + [\mathrm{CD}]_t = M$). ΔA values in the preparations were calculated by measuring the absorbance of Hal in the absence and presence of the corresponding concentration of CD. In the latter case, an equimolar aqueous solution of CD was used as a blank, to take into account its refractive index. Subsequently, $\Delta A[\mathrm{Hal}]_t$ was plotted for both CDs against the ratio (denoted as r)

$$r = \frac{[\mathrm{Hal}]_t}{[\mathrm{Hal}]_t + [\mathrm{CD}]_t}$$

3. Results and discussion

3.1. Phase solubility diagrams and calculation of the stability constants

The results obtained indicate that Hal is solubilized by both β-CD derivatives, with Meβ-CD contributing to greater drug solubility (Fig. 1). The solubility phase lines (isotherms) obtained (Fig. 1) from the equilibrium solubility studies were of the A_L type [6] for both Hal–CD complexes, suggesting that the complexes are readily soluble in water (for a 1:1 drug to CD stoichiometry). It appears that the solubilizing effect of Meβ-CD is greater than that of HPβ-CD (Fig. 1). This could be attributed to the higher aqueous solubility of the methylated derivative and/or a slightly better complexing ability of the derivative (also suggested by NMR and thermodynamic

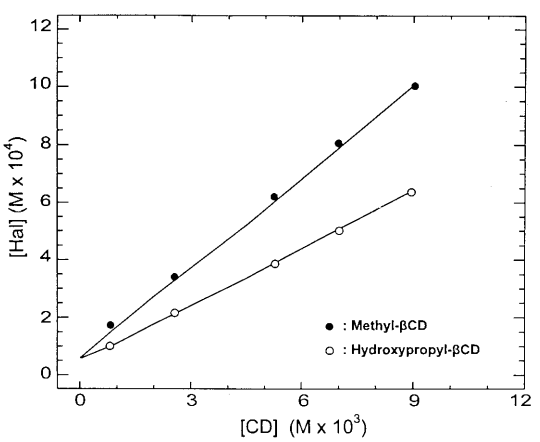

Fig. 1. Phase solubility diagrams of Hal–CD systems in water at 37°C.

Table 1
The effect of temperature on the K_{st} values of Hal–CD complexes

Temperature (K)	K_{st} (M^{-1})	
	Hal–Meβ-CD	Hal–HPβ-CD
300	2345	2112
310	1334	1123
320	1025	812
330	650	530

data; see below). The isotherms were also examined on the basis of linear and exponential curve fits according to which the correlation coefficients showed greater values for the linear type. In addition, the stability constants were calculated (Table 1) from the slope of the isotherms in the diagrams (Fig. 1) as,

$$K_{st} = \frac{\text{slope}}{S_o(1 - \text{slope})}$$

where S_o is the solubility of the drug alone (equal to the intercept of the diagram).

3.2. Determination of complex stoichiometries

To determine the stoichiometries of the complexes by the continuous variation method (Job plot) [11] UV spectra were obtained for a series of drug and CD mixtures in which the total initial concentrations of the two species were maintained constant ($[Hal] + [CD] = 0.17$ mM) but the ratio of initial concentrations ($r = [Hal]_t/([Hal]_t + [CD]_t)$) [12] varied between 0 and 1. If a physical parameter directly related to the concentration of the complex (for instance the absorbance A) can be measured under these conditions, and is then plotted as a function of r, the maximal value for this parameter will occur at $r = m/(m + n)$, where m and n are the Hal and CD proportions in the complex respectively (Hal_m:CD_n) [13]. This means that where complex stoichiometry is 1:1 ($m, n = 1$), the maximum value for the examined parameter will be reached at $r = 0.5$.

The calculated quantity $\Delta A[Hal]_t$ (where ΔA is the difference in absorbances between that of free Hal and the observed absorbance value for a given ratio r and $[Hal]_t$ denotes the total concentration of Hal) is proportional to the concentration of the complex and can be thus plotted against r. The continuous variation plot of $\Delta A[Hal]_t$ against r (Fig. 2) demonstrates that since the maximum has an r value of almost 0.5 in both cases, the complexes have a 1:1 stoichiometry.

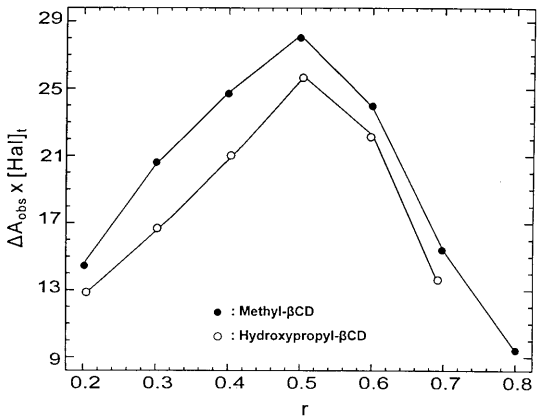

Fig. 2. Continuous variation plot (Job plot) of Hal–CD complexes.

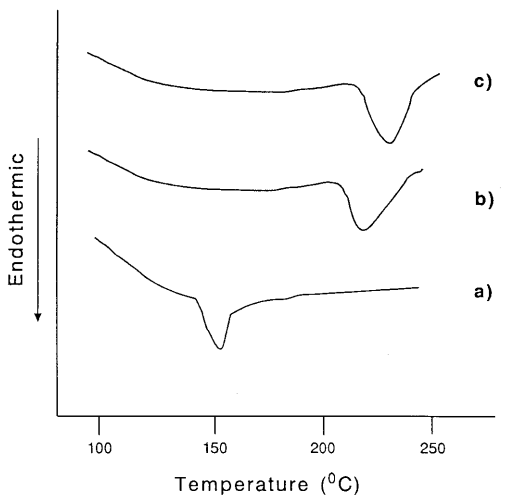

Fig. 3. DSC thermograms of free Hal (a), Hal–Meβ-CD and Hal–HPβ-CD complexes (b) and free Meβ-CD and HPβ-CD (c). The thermograms of free CDs and their complexes with Hal being nearly identical are shown as one thermogram in each case.

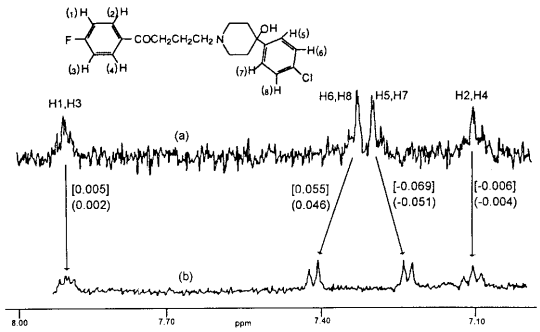

Fig. 4. Partial 500 MHz ^{1}H-NMR spectra of free Hal (a) and Hal–CD complexes (b) in 10% DCl. Numbers in brackets (Hal–Meβ-CD) and parentheses (Hal–HPβ-CD) denote the difference in chemical shifts $\Delta\delta(\Delta\delta = \delta_c - \delta_o)$ of the phenyl protons in the free (δ_o) and complexed state (δ_c). Negative values indicate upfield movement–shielding effect.

3.3. Characterization of inclusion complexes

Results (Fig. 3) from differential scanning calorimetry of the Hal–CD complexes are consistent with true complexation. Thus in the thermogram of the free Hal (Fig. 3a) there is one exothermic peak at 150°C, probably corresponding to the melting point of Hal. However, this peak disappears in the thermograms obtained with the two complexes and new ones appear at 220°C (Fig. 3b, pattern was identical for the two complexes), presumably reflecting thermic dissociation.

In the NMR studies, observed resonances for the soluble Hal–CD complexes were the time-averaged peaks obtained with the free CD and Hal and their inclusion complexes (fast exchange regime on the NMR time scale at 293 K). Formation of the inclusion complexes in aqueous solutions is evidenced basically by the modification of the NMR spectrum of Hal. As Hal is composed of two phenyl groups connected by a small carbon chain, a stoichiometry of 1:2 (one molecule of Hal with two molecules of CD, one for each phenyl group) is expected. However, results from the NMR study suggest that only the *p*-chlorobenzoyl moiety is involved in the inclusion process (both CDs). This is supported by the finding (Fig. 4) that the Hal phenyl group with the attached fluorine atom seems to remain outside the cavity, as it does not undergo any significant spectral changes. These results therefore indicate the formation of 1:1 inclusion complexes and support the stoichiometries calculated with the Job plot method. Failure of the fluorinated phenyl group to enter the CD cavity is probably due to its polar nature which renders the process of complexation unfavorable.

The NMR spectra of the two Hal phenyl groups in free and complexed forms (Fig. 4) obtained under the present conditions, show only shift changes of the corresponding signals. As there are no new peaks that could be assigned to the complexes as such, complexation of Hal with the CD appears to be a dynamic process with the Hal being in a state of fast exchange (relative to the NMR scale) between the free and included forms [14]. Thus, the exchange rate must exceed the reciprocal of the largest observed shift difference (in Hz) for any proton of the guest molecule.

3.4. Thermodynamic parameters of the Hal–CD systems

The values of enthalpy (ΔH_0) and entropy (ΔS_0) for the stability constants of the CD complexes with Hal were determined from the linear plots of $\ln K_{st}$ versus $1/T$ (reciprocal temperature in K) according to the integrated form (Fig. 5) of Van't Hoff equation [15],

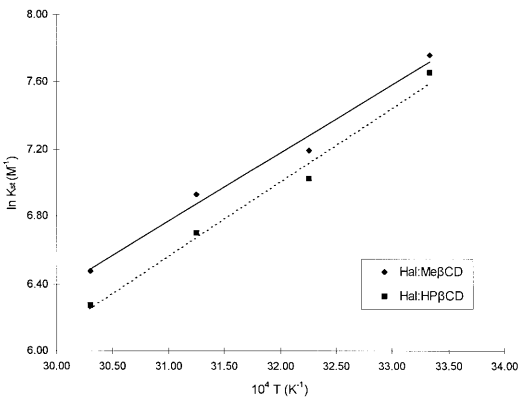

Fig. 5. Van't Hoff plots for the stability constants of Hal–MeβCD and Hal–HPβCD complexes.

Table 2
Thermodynamic parameters (enthalpy and entropy) for the Hal–CD complexes

Complex	ΔH_0 (kcal mol^{-1})	ΔS_0 (cal mol^{-1} K^{-1})	R
Hal–Meβ-CD	−8.1	−11.6	0.985
Hal–HPβ-CD	−8.8	−14.3	0.986

$$\ln K_{st} = -\frac{\Delta H_0}{R}\frac{1}{T} + \frac{\Delta S_0}{R}$$

The enthalpy of the complexes formed between Hal and either of the two β-CD derivatives is exothermic (negative values in Table 2). Such negative values for enthalpy may be due to enhanced Van der Waals interactions between the guest Hal molecules and the CD cavities, hydrogen bonding between the guest and groups within the CD cavity or to changes in the degree of aggregation of water associated with the cyclodextrin molecules on complex formation [6]. In the latter case, 'high-enthalpy' water molecules can be readily displaced [6] by less polar guest molecules or portions thereof to combine with the bulk of the solvent and thus increase the number of energetically favourable solvent–solvent hydrogen bonds. In the present study, however, the greater degree of complexation (Table 1) observed with Meβ-CD as compared with that of HPβ-CD suggests that such formation of additional hydrogen bonds between Hal and CD cannot be the main driving force for Hal complexation since, of the two derivatives, Meβ-CD has fewer free hydroxyl groups within the cavity: the substitution of the hydroxyl groups of β-CD with the methoxyl groups promotes the hydrophobic character of Meβ-CD (compared to that of HPβ-CD) and thus enhances the hydrophobic binding of Hal.

In contrast, it is well known [6] that entropy changes (ΔS_0) after complexation are mainly derived either from the loss of degree of freedom (both translational and rotational) by the guest that is due to the association of the complex forming molecules or from a change in the ordering of the solvent molecules. The overall entropy change is the net result of these two opposite effects. The negative entropy changes observed (Table 2) with the Hal complexes indicate that the reduction of the translational and rotational degrees of freedom due to the complexation has a greater effect on such changes than solvent disordering. Values in Table 2 also indicate that complex formation with the Meβ-CD is a more favoured event than with HPβ-CD, possibly because of a reduced restriction of movement of the Meβ-CD molecules in which the methyl groups are less bulky than the hydroxypropyl groups of the HPβ-CD.

In conclusion, through complexation with the two β-CD derivatives, the aqueous solubility of Hal has been improved substantially (up to 20-fold) in neutral aqueous solutions. Both CD derivatives were shown to provide complexes that are readily soluble in water with a complex drug to CD stoichiometry of 1:1. Moreover, both complex formations occur under thermodynamically favoured conditions, with an overall complexing ability that is slightly greater for the Meβ-CD derivative. These findings could contribute to new, non-acidic formulations of Hal.

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