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The formation of an inclusion complex of methocarbamol with hydroxypropyl-β-cyclodextrin: the effect on chemical stability, solubility and dissolution rate

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Abstract

The inclusion complex of the hydrophobic drug methocarbamol (ML) with hydroxypropyl-β-cyclodextrin (HPβCD) was prepared and characterized in the solid state and in aqueous solution. The prepared complex was studied by chromatographic, spectral and phase solubility methods to determine its structure, solubility, chemical stability and dissolution rate. Host-guest interactions in aqueous solution were studied by proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) and in the solid state by differential scanning calorimetry (DSC) and infrared spectroscopy (IR). The stoichiometry of the isolated complex was determined by reversed phase high pressure liquid chromatography (RP-HPLC), ^{1}H-NMR spectroscopy and elemental analysis. The solubility and dissolution rate of ML in free and complexed form were examined in aqueous solution. The stability constant of the complex was determined by the classical solubility techniques. The chemical stability of free and complexed ML, in buffered solution at pH 7.4 and at two different temperatures, 37 and 60°C, was monitored using an HPLC method and resulted in a 2-fold increase in the stability of complexed ML compared to free ML. © 1997 Elsevier Science B.V.

Keywords: Cyclodextrin; Methocarbamol; Stability; Solubility; Dissolution

1. Introduction

Methocarbamol (ML), a carbamate structural analogue of the aryl glycerol ethers, is a centrally active muscle relaxant used in the symptomatic

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treatment of musculosceletal disorders and may be given orally or by injection. ML has limited water solubility and undergoes degradation in aqueous solutions under neutral and alkaline conditions. Previous work (Pouli et al., 1994) has shown that ML hydrolysis to the corresponding Diol (DL) (Fig. 1, III) was mainly proceeded through the formation of ML isomer, isomethocarbamol (IML, II) and the degradation rate at constant pH and temperature, followed pseudo first order kinetics. To overcome these problems the USP suggested the utilisation of solubiliser and pH values in the range of 3.5–6 for injected formulations. In order to achieve new, water soluble formulations of the drug with improved chemical stability, the complexation with cyclodextrins was examined.

Cyclodextrins (CDs) are cyclic oligosaccharides consisting of six, seven or eight D-glycopyranose units (α-, β- and γ-cyclodextrin) linked by a (1–4) glycosidic bonds. They have a torus-shaped, apolar, electron rich hydrophobic cavity, with internal cavity sizes of 0.5, 0.6 and 0.8 nm respectively. The hydroxyls of this macromolecule oriented to the exterior create sites available for hydrophobic interactions. Initially, the CD cavity is occupied by water molecules. The presence of hydrophobic molecules dispersed in the aqueous media leads to noncovalent inclusion complex formation through displacement of the included water molecules by the hydrophobic ones. In aqueous solutions of the complexes, free drug molecules are in equilibrium with the drug molecules entrapped within the cavity.

In recent years, chemically modified cyclodextrins, such as HPβCD, have been used widely in pharmaceutical formulations in order to improve the physicochemical properties of various drugs. It has been shown that the chemical stability and aqueous solubility of drugs can be optimized by forming inclusion complexes with CDs (Duchêne, 1991; Lôftsson and Brewster, 1996; Loukas et al., 1995; Szejtli, 1982). HPβCD has very low toxicity by the parenteral route and no adverse effects have been observed in humans (Rajewski and Stella, 1996; Carpenter et al., 1995). In this study, an HPβCD complex of ML has been prepared by the freeze drying method. The complex has been investigated to probe any possible optimisation of the pharmaceutical parameters of the drug.

2. Experimental section

2.1. Materials

ML and HPβCD (degree of substitution 0.8 and average molecular weight of 1500) were purchased from Aldrich Chemical Company. Organic solvents were HPLC grade, all other chemicals were reagent grade and were used without further purification. The water used was deionised and filtered by a MilliQ-Plus water purifying system.

2.2. Methods and instrumentation

The structure of the complex was investigated in aqueous solution by using ^{1}H-NMR spectroscopy. The NMR spectra were recorded at 200 MHz on a Bruker AC 200 instrument using D_2O as solvent. Typical conditions were 16 K data points with zero fitting sweep width 1.4 KHz, giving a digital resolution of 0.34 Hz point^{-1}, pulse width 2 ìs (90° pulse 5.5 ìs) and acquisition time 2.9 s. Gaussian enhancement was used for the displayed spectra (GB = 0.2, LB = −2).

Differential scanning calorimetry (DSC, Perkin Elmer DSC7) measurements were performed using samples of 1.5–5.25 mg accurately weighed into non-hermetically sealed aluminium pans. The heating rate was 10°C min^{-1}, a nitrogen purge

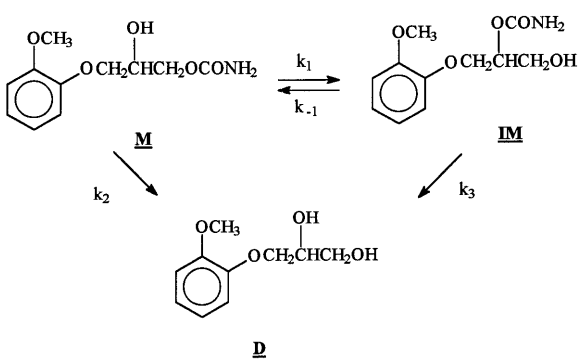

Fig. 1. Chemical structure of ML and its degradation products.

was maintained throughout runs and base line optimisation was performed before each run.

The IR spectra (Perkin Elmer 883) were recorded using nujol mulls or potassium bromide disks at a pressure of 30 kg cm^{-1}.

The quantitative assay for ML was performed on a Waters (Milford, MA) liquid chromatography instrument consisting of a pump, Model 590 with a 10 μm reversed phase 3.9×150 mm C_{18} column, a Rheodyne 717S injector, fitted with a 20 μl loop, a UV detector (Lambda-Max, Model 481) set at 274 nm and an integration system (Waters Basline 810). The degassed mobile phase was water:acetonitrile 9:1 (v/v) solution, containing 1% acetic acid, this was vacuum filtered through a 0.45 μm pore-size nylon membrane filter. Under these conditions ML was separated from its degradation products. All runs were performed at room temperature, the flow-rate was maintained at 2.4 ml min^{-1}, with a detector sensitivity 0.05 AUSF and the pressure at 740–760 psi.

2.3. Preparation of ML:HPβCD complex

The preparation of ML:HPβCD complex was carried out according to the freeze drying method (Loukas et al., 1994) as follows: Equimolar quantities (1×10^{-2} M) of ML and HPβCD were combined and the resulting aqueous solution was stirred for 24 h at constant temperature (40°C). The above solution was then frozen by immersion in liquid nitrogen and freeze dried for 24 h. The amounts of the ML degradation products were negligible. The experiment was repeated by using ML diluted in a small volume of methanol. The obtained solution was stirred for 5 h and the organic solvent was removed by using a stream of nitrogen. After the evaporation of methanol, the solution was freeze dried according to the above mentioned procedure.

2.4. Phase solubility technique

A solubility phase diagram was performed according to the method of Higuchi and Connors (1965). Accurately weighed excess amounts of ML, 732 mg (6×10^{-2} M) portions were added to 50 ml sealed glass vials, containing aqueous solutions of increasing HPβCD concentrations (ranging between $0.001-4 \times 10^{-2}$ M). The vials were immersed in a thermostatic shaking (150 strokes/min) water bath at 28 ± 0.5°C for 250 h. Preliminary experiments indicated that 250 h provided sufficient time for the system to reach equilibrium. After equilibrium had been reached, the content of each vial was filtered (0.45 μm pore-size Millipore membrane filter). The first 25% of the filtrate was discarded to avoid any potential loss of the drug, because of adsorption by the filter unit and the subsequent filtrate was collected. All procedures were conducted at the test temperature to avoid any precipitation of the drug in the vehicle. The filtrate was appropriately diluted and ML concentration was determined by HPLC.

2.5. Stability studies

The kinetic studies of the ML degradation reaction were performed in KH_2PO_4, K_2HPO_4 buffer solutions with pH 7.4 (± 0.1) at constant temperatures of 37 and 60°C. The ML hydrolysis was monitored using the HPLC assay described above.

2.6. Dissolution rate studies

The dissolution rate of 120.5 mg powder samples of ML or equivalent quantity of ML:HPβCD complex was determined by the paddle method using the USP dissolution apparatus, in distilled water, at 37 ± 0.5°C. Rotation was set at 50 rpm. The concentration of ML was determined by HPLC. Each experiment was performed at least three times and the mean value was calculated in each case.

3. Results and discussion

The formation of the ML:HPβCD complex resulted in relatively good yields, despite some degradation problems (Fig. 1). The degradation of ML can be eliminated by stirring the solution at a temperature lower than 40°C.

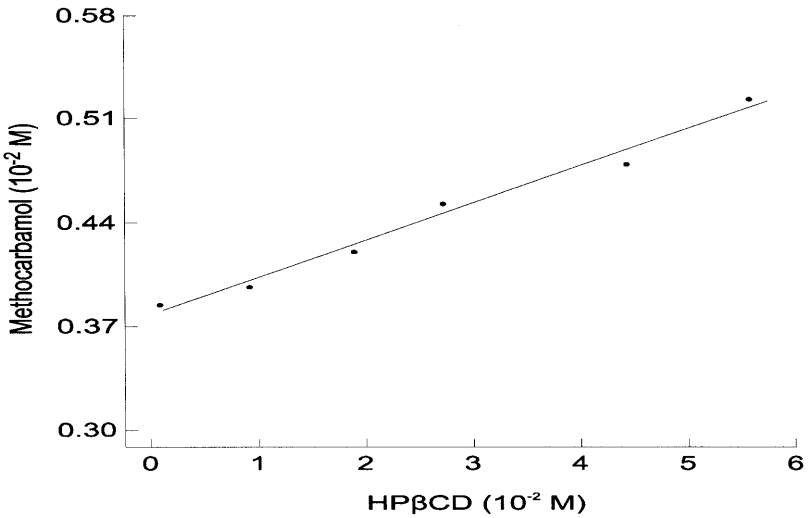

Fig. 2. Phase solubility profile for ML and HPβCD in water at 28°C.

3.1. Phase solubility studies

The phase solubility profiles for the complex formation between ML and HPβCD are presented in Fig. 2. These diagrams show that the aqueous solubility of the drug increases linearly as a function of HPβCD concentration. It is clear that this solubility diagram can be classified as the A_L type according to Higuchi and Connors (1965) indicating the formation of the ML:HPβCD inclusion complex. The apparent stability constant of the complex at 28°C, was calculated to be 17.8 M^{-1}. This was determined from the slope of the phase solubility line (Fig. 2) as follows: $K_{1:1} = \text{slope}/S_0\ (1\text{-slope}) = 17.8\ M^{-1}$, where S_0 is the solubility of the pure drug at the same temperature ($S_0 = 0.375$).

3.2. Characterisation of the ML:HPβCD complex in the solid state

3.2.1. Differential scanning calorimetry

DSC thermographs of ML from 0 to 240°C (Fig. 3) exhibit a sharp endothermic peak at 102.73°C corresponding to its melting point (enthalpy change = 160 J/g). The DSC data for HPβCD (Fig. 3) shows an endothermic peak at 90–100°C corresponding to its dehydration. However, for the complex (Fig. 3), where the included water molecules have been totally or partly replaced by the drug, neither of these two peaks were observed, indicating that the crystal lattice of the drug has been disturbed. For the HPβCD complex the above two peaks were replaced by a weak broad endotherm, spreading between 46 and 90°C with an enthalpy change of 0.94 J/g. The physical mixture of ML:HPβCD showed the retention of a melting peak for the drug superimposed on the water loss associated with the cyclodextrin. A further endotherm is seen after the melt of ML which must be indicative of an interaction formed in the mix at these elevated temperatures.

3.3. Infrared spectroscopy

The functional groups of ML involved in the complexation were investigated by IR spectroscopy. Strong absorption peaks are seen at 1684 and 1695 cm^{-1} which can be attributed to ML carbonyl group stretching vibration. This group has the natural tendency to dimerize through the formation of intermolecular and hydrogen bonds. In the spectra of the complex this band was shifted towards higher frequencies: 1708 and 1720 cm^{-1}, suggesting that after the formation of the 1:1 complex existing bonds were broken. The magnitude of the shift of wavenumber

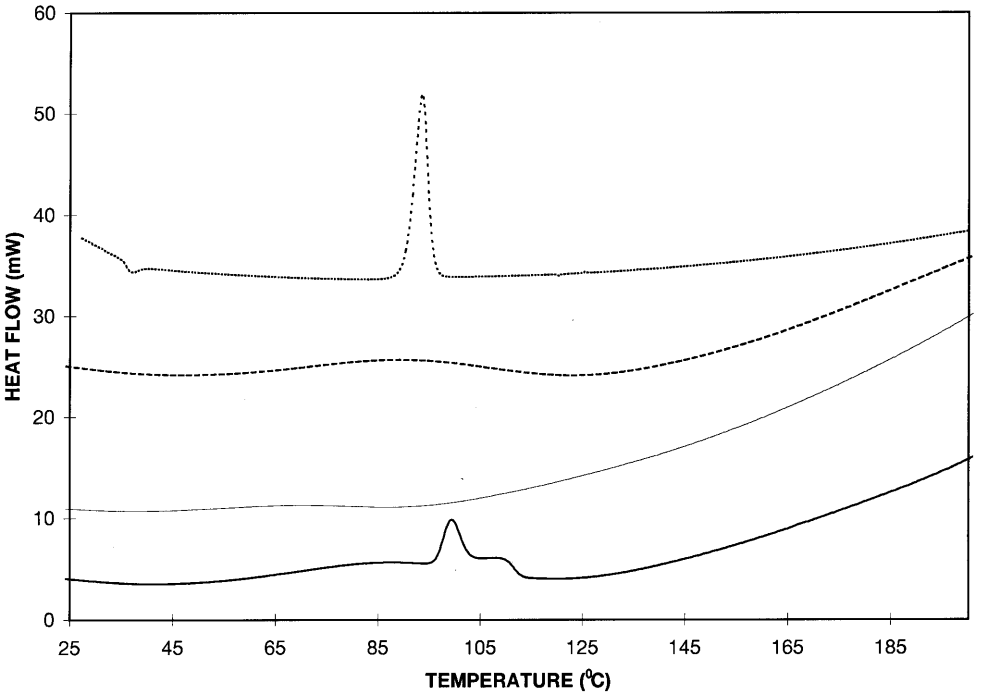

Fig. 3. DSC thermographs. From top to bottom ML; HPβCD; ML:HPβCD complex prepared by the freeze drying method; ML:HPβCD physical 1:1 mix (displacement of traces has been made to allow comparison).

given by the same group is of the range of 24 cm^{-1} (Table 2). However, there is no change in the spectra of equimolar ML and CD physical mixtures, confirming that in this case these hydrogen bonds remain unchanged.

3.4. Characterization of the ML:HPβCD complex in aqueous solution

The complex formation is expected to induce shifts in the resonance of the CD protons, especially of protons directed towards the interior of the CD cavity ($H-C_3$ and $H-C_5$). The resonance modifications of the NMR spectra of both guest and host molecules provides evidence of complexation (Table 1). In the case of the ML:HPβCD complex, protons of the maltose subunits showed upfield shifts due to the ring effect of the aromatic guest. The sharp singlet due to the methoxy group protons of ML, appearing at 3.86, undergoes a downfield shift following complexation. These methoxy group protons were near the wide edge of the cyclodextrin. This location was expected because of the ortho position of the phenyl ring substituents. However, several of the peaks consisting the multiplet corresponding to the propan–2–ol side chain protons of ML molecule, show only slight variations in the range of 0.011–0.012 ppm, indicating a position near the edge

Table 1
Wavenumber shift of the functional group of ML prior and after complexation of HPβCD in IR spectra

	Wavenumber α cm^{-1}	Wavenumber β cm^{-1}	Shift cm^{-1}
C=O	1684	1708	24
	1695	1719	24

α: wavenumbers corresponding to the pure ML and the physical mixture; β: wavenumbers corresponding to ML:HPβCD.

Table 2
Chemical shifts δ (ppm) of protons of ML and HPβCD in the free and complexed forms

Proton[a]	$\delta_{o\ (free)}$	$\delta_{c\ (complex)}$	$\Delta\delta_{(\delta c-\delta o)}$
Methocarbamol			
CH_3	3.8559	3.8788	+0.0229
Aryl	7.0118	6.9868	−0.0250
Aryl	7.0285	7.0039	−0.0246
Aryl	7.0451	7.0174	−0.0277
Aryl	7.0670	7.0362	−0.0308
HPβCD			
CH_3	1.1346	1.1031	−0.0315
CH_3	1.1661	1.1344	−0.0317
H	3.6035	3.5717	−0.0312
H	3.8768	3.8226	−0.0542
H	3.9997	3.9730	−0.0267
Anomeric	5.0894	5.0381	−0.0513
Anomeric	5.2628	5.21264	−0.0502

[a] Only the protons that show chemical shift change.

(Komiyama, 1989). The NMR spectra also consists of sets of signals assigned to the phenyl group protons of ML. These protons are affected by the complexation and exhibit significant displacements in the range of 0.025–0.031. All the above are indicative of the incorporation of the phenyl group of ML in the CD cavity.

3.5. The stoichiometry of the HPβCD inclusion complex

The stoichiometry was measured by ^{1}H-NMR, HPLC and elemental analysis. Digital integration of selected ^{1}H-NMR signals from the host and the guest molecule protons of the soluble complex, provided direct access to the stoichiometry coefficient. In the case of HPLC, an accurately weighed quantity of complex was dissolved in distilled water, the sample was analysed and the concentration of the guest (ML) in the chromatogram was calculated by reference to a calibration curve. The results were in agreement with those calculated by ^{1}H-NMR and elemental analysis. In all cases the calculated stoichiometry coefficient was found to be 1:1.

3.6. Stability studies

The degradation rate constant of ML at 60°C decreased from 5.3×10^{-3} h^{-1} (free ML) to 2.8×10^{-3} h^{-1} (complexed ML) while at 37°C decreased from 3.5×10^{-4} h^{-1} to 1.6×10^{-4} h^{-1}. Thus the rate of hydrolysis of ML is reduced by almost 50% when complexed in HPβCD. This is due to the fact that the susceptible moiety of ML was included in the hydrophobic cavity of the HPβCD and was protected from the attack of the aqueous buffered media.

3.7. Dissolution studies

The dissolution data are shown in Fig. 4. It is evident that the HPβCD complex of ML exhibits faster dissolution than free ML. The HPβCD complex dissolved in 60 s, whilst lyophilized ML and the mechanical mixture of ML and HPβCD in equimolar quantities were completely dissolved in 7 and 15 min, respectively. The enhancement in the dissolution rate of the complex could be explained from the enhanced solubility of the drug due to the complexation.

4. Conclusion

The complexation of ML with HPβCD enhances both the solubility and the dissolution of the drug in aqueous media. The described HPβCD complex of ML could be used in new, nonacidic and water soluble formulations of ML.

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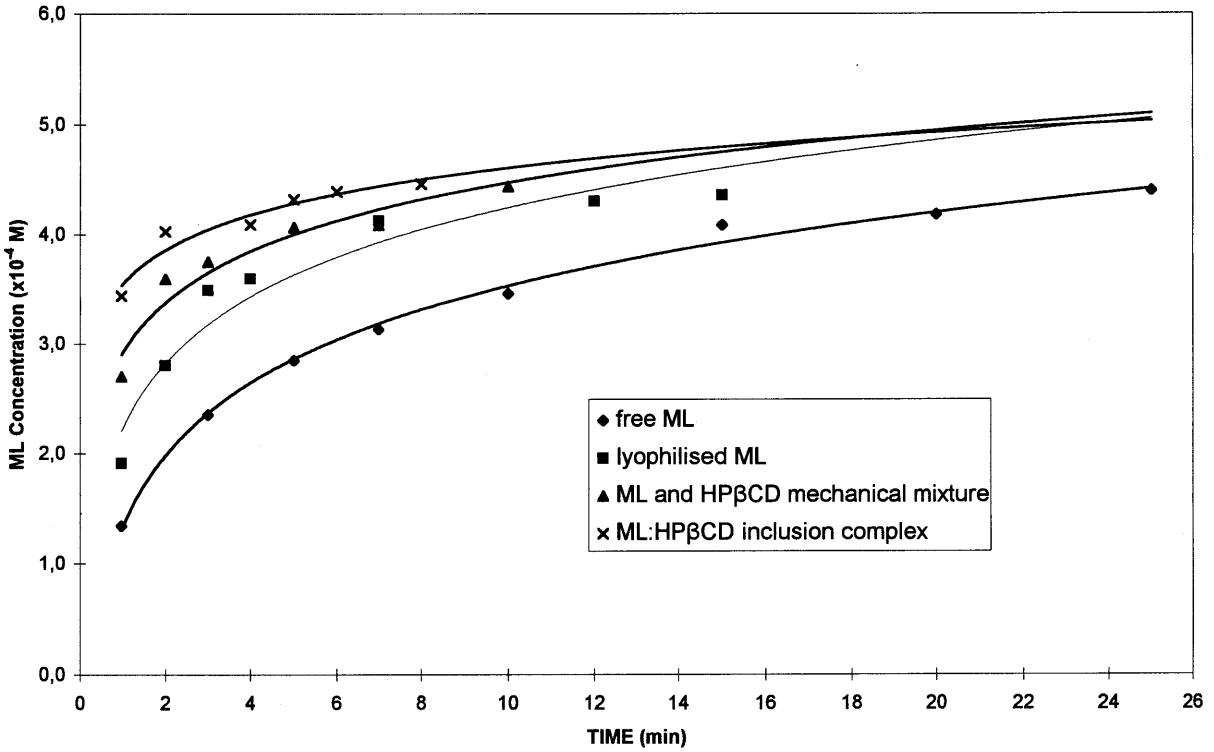

Fig. 4. Dissolution profiles of ML in free and complexed form and as a mechanical mixture with HPβCD.

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