DICLOFENAC - ß - CYCLODEXTRIN INCLUSION IN SOLUTION PROTON MAGNETIC RESONANCE AND MOLECULAR MODELLING STUDIES

DARRYL V. WHITTAKER, LAWRENCE J. PENKLER, LUÉTA A. GLINTENKAMP, M.C. BOSCH VAN OUDTSHOORN AND PHILLIPUS L. WESSELS†

Druggists Group research, South African Druggists Limited, P.O. Box 4002, Korsten, 6014, Port Elizabeth, South Africa
†Department of Chemistry, University of Pretoria, 0002, South Africa

ABSTRACT

The nature of the inclusion complex of the NSAID [2-[(2,6-dichlorophenyl) amino]phenyl]acetic acid (diclofenac) with ß-cyclodextrin and 2-hydroxypropyl-ß-cyclodextrin was studied by selected 1D and 2D NMR experiments and by molecular modelling studies using Hyperchem® software. The results indicate that simultaneous inclusion of both rings occurs even at low drug/CD ratios giving rise to multiple equilibria in solution (two isomeric 1:1 complexes and a 1:2 complex). Continuous variation plots are supportive of 1:1 stoichiometry indicating that isomeric 1:1 complexes predominate in the range of concentrations studied.

1. INTRODUCTION

The aqueous solubility of diclofenac is pH dependant increasing substantially from < 0.02 mg/mL at pH 2.0 to >16 mg/mL at pH 7.0 due to ionization of the acetate functionality (pKa ~ 4). At neutral pH in 2-hydroxypropyl-ß-cyclodextrin solutions, the solubility of diclofenac is enhanced by the combined effect of ionization and inclusion complexation permitting preparation of stable aqueous solutions containing 25mg/mL, suitable for intravenous and intramuscular administration[1]. The net solubility enhancement of the drug is therefore largely a consequence of increased saturation solubility of the drug due to ionization, with a lesser contribution from inclusion complexation of the ionized (and unionized) species. However, the complex stability constant decreases substantially with increasing pH (from greater than 1500 M^{-1} at pH 2.0 to less than 100 M^{-1} at pH 7.0)[2], suggesting that the ionized drug is less complexable than the free acid.

Journal of Inclusion Phenomena and Molecular Recognition in Chemistry **25**: 177–180, 1996.

Thus, in order to better understand the nature of inclusion complexation in aqueous solution, selected proton magnetic resonance experiments combined with energy minimization calculations were performed at neutral pH.

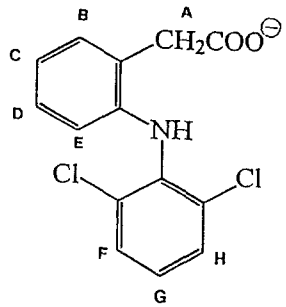

Figure 1. Structure and proton notation of Diclofenac

2. MATERIALS AND METHODS

2.1 NMR spectroscopy

Proton NMR spectra were recorded on a Bruker AMX 500 spectrometer operating at 500.13 Mhz (303K). Chemical shifts are given relative to external TMS. 25 mM solutions of diclofenac in BCD and HPBCD and a lyophilised (1:1) complex were prepared in D_2O (pD = 7.00). Resonance assignments of diclofenac, BCD and HPBCD were obtained from the 2D experiments and correlated with published data[3]. ROESY experiments were performed using a 150 ms spin-locking time and were transformed to a 1K x 1K data matrix. Continuous variation plots were obtained from solutions of the drug and CD where the total concentration was kept at 10 mM and the ratio of the two components varied between 0 and 1.

2.2 Computational Methodology

Calculations were performed using the Hyperchem® program running on an IBM PC. The molecular structures of diclofenac and BCD were generated by manual techniques. In the case of BCD, improper torsional constraints were imposed using the atoms $(C5 - C4)_n$ $(C1 - O5)_{n-1}$, where the value of the improper torsion was constrained about 0° with a force constant of 16 kcal.deg $^{-1}$to induce C7 symmetry.

Geometry optimisation was performed *in vacuo* using the MM+ force field with the Polak Ribierre conjugate gradient method and termination condition of 0.01 kcal.mol^{-1}. In all calculations, charges were omitted to prevent exaggerated electrostatic interactions resulting from lack of solvent shielding effect. The optimised CD structure was in agreement with experimental data derived from X-ray analysis[4].

3. RESULTS AND DISCUSSION

As expected, 1D NMR spectra of solutions of diclofenac and CD contained only one set of resonances for each proton or group of equivalent protons confirming that the fast exchange process prevails. Significant *upfield* shifts for the internally oriented H3' and H5' protons of the CD, attributable to the inclusion of an aromatic substituent were observed. However, *downfield shifts* for aromatic protons of *both* rings (TABLE 1) are suggestive of bimodal inclusion. Further evidence of bimodal inclusion complexation was obtained from ROESY spectra of solutions of the complex. Intermolecular cross-peaks were observed between certain aromatic protons from both rings of the drug and the 3' and 5' protons of BCD and HPBCD, even at low drug/CD ratios, indicating that three different complexes are possible in solution, namely two isomeric 1:1 complexes and a 1:2 complex.

TABLE 1. Proton NMR chemical shift data (500 MHz) at 303 K (D2O, ext TMS) for Diclofenac and an equimolar 1:1 solution of Diclofenac (5mM) and ß-CD (5mM)

Diclofenac	Free	Complex	Δ δ	Nature of shift
Ha	2.989	3.027	-0.038	*Downfield*
Hb	6.597	6.609	-0.012	*Downfield*
Hc	6.299	6.306	-0.007	*Downfield*
Hd	6.448	6.475	-0.027	*Downfield*
He	5.811	5.790	+0.021	*Upfield*
Hf,h	6.817	6.848	-0.031	*Downfield*
Hg	6.485	6.475	+0.010	Upfield
ß-Cyclodextrin				
H1'	4.385	4.361	+ 0.024	*Upfield*
H2'	2.965	2.939	+ 0.026	*Upfield*
H3'	3.275	3.208	+ 0.067	*Upfield*
H4'	2.897	2.869	+ 0.028	*Upfield*
H5'	3.175	3.100	+ 0.075	*Upfield*
H6,6'	3.192	3.183	+ 0.009	*Upfield*

In the solid state, the diclofenac/BCD complex crystallises in a unique hexagonal crystal system with the phenylacetate ring preferentially included due to the bulkiness of the dichlorophenyl substituent[5]. However, in solution, the NMR data clearly shows that the dichlorophenyl substituent is also complexed. The equivalent aromatic protons Hf,h on the more hydrophobic dichlorophenyl ring showed slightly more intense ROE cross peaks to H3' and H5' and slightly larger downfield shifts in the 1D spectra than the protons Hd and He on the phenylacetate ring, suggestive that it may be preferrentially included. No cross peaks were observed between Hb and Hc on the phenylacetate ring. and the H3' and H5' protons of BCD. Refer to Figure 1.

Continuous variation plots constructed from proton chemical shift data indicate that complex stoichiometry is predominantly 1:1, however slight skewing of the curves for the diclofenac protons to the left of $r = 0.5$ suggests the presence of 1:2 complexes ($r \sim 0.4$-0.5).

Further insight into the geometry of the proposed complex(es) was obtained from molecular modelling studies. Several rigid body docking experiments were carried out to position diclofenac in the BCD cavity ensuring non-violation of van der Waals contacts. Four different binary complexes were geometry optimized with inclusion of phenylacetate and chlorophenyl rings respectively, and seven different ternary complexes with simultaneous inclusion of both aromatic rings were similarly treated. Optimized conformations were in close agreement with NMR chemical shift and ROE data.

4. CONCLUSION

There is evidence that complexation of both aromatic substituents of diclofenac occurs in solution at neutral pH despite the low complex stability constant attributable to the ionizable acetate functionality.

REFERENCES

[1] Penkler, L.J. *et al* European Patent. 94308690.0, South African Druggists Ltd.

[2] Orienti, I., Fini, A., Bertasi, V., and Zecchi, V, Inclusion Complexes between Non Steroidal Antiinflammatory Drugs and ß-cyclodextrin, *Eur J Pharm. Biopharm* 37 (1991) 110-112.

[3] Florey, K, *Analytical Profiles of Drug Substances*, Volume 19, Academic Press Inc, 1990.

[4] Lindner, K. and Saenger, W. *Carbohydr. Res.* 99 (1982), 103-115.

[5] M.R. Caira, V.J. Griffith, L.R. Nassimbeni and M.B. van Oudtshoorn, Synthesis and X-Ray Crystal Structure of ß-cyclodextrin. Diclofenac Sodium Undecahydrate, a ß-CD Complex with a Unique Crystal Packing Arrangement, *J. Chem. Soc. Chem. Commun* (1994) 1061-1062.