INFLUENCE OF DEGREE AND PATTERNS OF SUBSTITUTION ON THE INCLUSION PROPERTIES OF ETHYLATED β-CYCLODEXTRINS

D. WOUESSIDJEWE[1], V. LEMESLE-LAMACHE[1], D. DUCHENE[1], B. PERLY[2]

[1]Laboratoire de Physico-Chimie, Pharmacotechnie et Biopharmacie, URA CNRS 1218, Faculté de Pharmacie, Université de Paris-Sud, 92290 Châtenay Malabry, France

[2]CEA, DRECAM, Service de Chimie Moléculaire, Centre d'Etudes Atomiques, 91191 Gif sur Yvette, France

ABSTRACT

Different batches of ethylated derivatives of β-cyclodextrins (Et-βCD) obtained by various synthetic routes were used as host molecules to prepare inclusion compounds of salbutamol. Previous results showed that these complexes were suitable to achieve variable sustained-release behaviour of salbutamol. The mechanism of sustained-release was related to the inclusion capacity and physicochemical properties of Et-βCD. Among the various analytical techniques carried out to characterize them, high resolution NMR afforded relevant information in terms of degree and patterns of subtitution, which could explain the differences observed between CD derivatives.

1. INTRODUCTION

The chemical modifications of natural cyclodextrin by a regioselective substitution of the hydroxyl groups located on the C2, C3 and C6 positions is very complicated to achieve, due to the differences in reactivity between the hydroxyl groups and steric factors of the cyclodextrin torus [1]. Two different strategies of synthesis, varying by the choice of reagent and operating condition, were used to obtain Et-βCD .These CD derivatives were successfully used to achieve inclusion compounds of a drug model, salbutamol. The complexes were submitted to dissolution assays *in vitro,* and the results showed variable sustained release of salbutamol [2]. It was obvious that these differences in behaviours of the complexes could be related to the properties of the Et-βCD. A combination of many characterization methods, such as solubility study, X-ray diffraction patterns and electrospray ionization mass spectrometry was carried out on the CD derivatives [3].

This paper deals with high resolution ^{1}H NMR and ^{13}C NMR of ethylated β-cyclodextrins, which appeared as an ultimate tool allowing the determination of the homogeneity of CD derivatives from batch to batch.

2. MATERIALS AND METHODS

2.1. Materials

Four batches of ethylated β-cyclodextrin were used in this study. Batch N1 was purchased from CYCLOLAB (Budapest, Hungary). Briefly, this product was synthetized by reacting dried βCD with ethyl iodide in alkaline-DMSO media at room

temperature. The final Et-βCD was separated from the reactive medium by a series of crystallizations in different media.

Batches N9A, N10 and N10A were generous gifts from ORSAN (Les Ulis, France). The synthetic pathway consisted in ethylation of βCD using an excess of ethyl sulphate in strong alkaline organic medium. Ethylated β-cyclodextrins were isolated from the reactive medium using suitable procedures. These three different batches were prepared with a view to obtaining various patterns of substituted βCD derivative.

2.2 Methods

All NMR experiments shown here were performed using a dual ^{13}C-^{1}H probe on a Bruker AMX 500 spectrometer operating at 500.13 and 125.77 MHz respectively for ^{1}H and ^{13}C. The length of 90° pulse was ca 11 and 6 μs for ^{1}H and ^{13}C. All spectra were collected in deuterium chloroform at 298 K.

3. RESULTS AND DISCUSSION

3.1. Determination of degree of substitution

^{1}H NMR spectra of ethylated β-cyclodextrins, batches N1 and N9A, are displayed in Figure 1. Comparison of the integration of the signals in the 1.0 to 1.5 ppm spectral region (ethyl group only) and of 3.0 to 4.5 ppm region (all signals from glucose units except H-1 and methylene groups) directly provides the DS, that is the number of ethyl chains by glucose unit. Although the spectra appear rather different, they give a very similar DS of 2, confirming diethyl βCD stuctures. However, only the sample N1 is consistent with a single symmetrical structure and correspond to the expected 2,6 diethyl βCD. The sample N9A (as N10 and N10A) reveal a large molecular heterogeneity induced by the variations on the substitution positions.

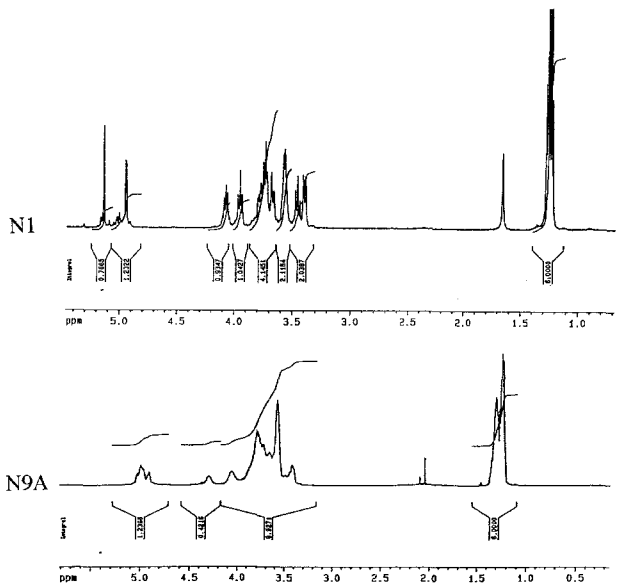

Fig. 1 ^{1}H NMR spectra of Et-βCDs (samples N1 and N9A) in $CDCl_3$ at 298 K

3.2. Complete assignment of ^{1}H and ^{13}C spectra

Only the sample N1 was subjected to this complete procedure. Figure 2 shows the ^{1}H NMR spectrum achieved by a double-quantum correlation experiment. All the signals were assigned subsequently starting from the ^{1}H signals of anomeric protons at 4.9 ppm.

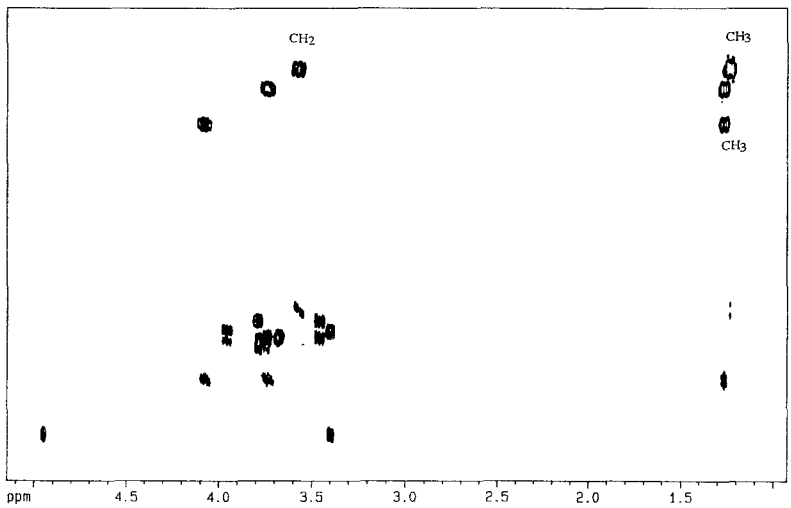

Fig. 2 Double-quantum correlation experiment for sample N1 performed in $CDCl_3$ at 298 K

Figure 3 displays the ^{13}C NMR spectrum of sample N1. The signals were assigned from the heteronuclear correlation procedure starting from information gathered for protons (see Figure 4).

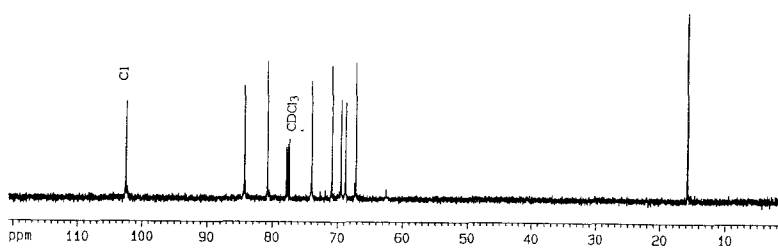

Fig. 3 ^{13}C NMR spectrum of sample N1 in $CDCl_3$ at 298

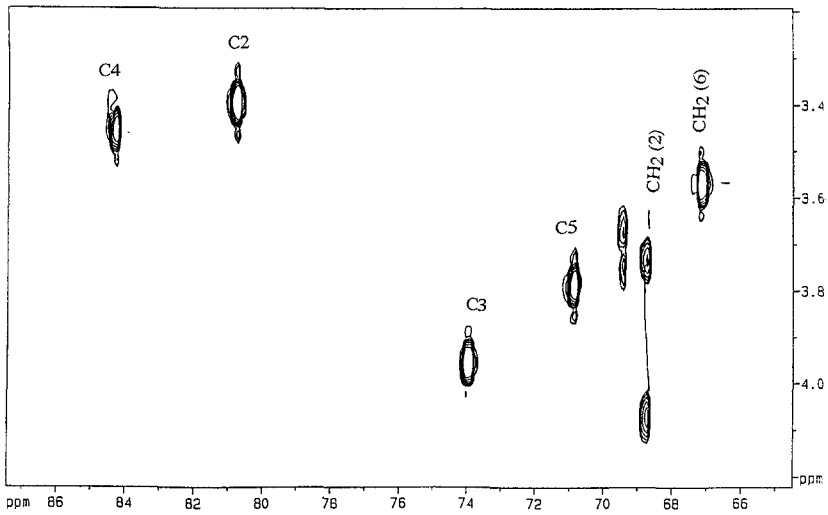

Fig. 4 ^{13}C-^{1}H 2D correlation experiment for sample N1

For the other samples N9A, N10 and N10A, the heterogeneity of signals from C-6, and the presence of a signal at 4.35 ppm suggest that ethylation may occur on the 2, 3 and 6 positions. However, from the standpoint of pharmaceutical use, these heterogeneous derivatives, diethyl β-cyclodextrins, revealed interesting properties on the sustained release of salbutamol.

CONCLUSION

Different batches of ethyl β-cyclodextrins obtained from various synthetic routes were used to achieve inclusion compouuds of salbutamol. The variable sustained-release behaviour of the complexes were related to the physicochemical properties of cyclodextrin derivatives. High resolution ^{1}H NMR and ^{13}C NMR were used to characterize these diethlyl β-cyclodextrins in terms of average substitution ratio and location of substituents on the CDs glucose units. This approach is more realistic and very useful to understand the CD properties from batch to batch.

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