nanalysis

USP-NF: Hydroxypropyl Betadex; Ph. Eur.: Monograph 1804

Molar Substitution Determination in Hydroxypropyl Betadex According to USP-NF/Ph. Eur. (1804) Monograph Method Using ^{1}H Benchtop NMR

Background

Cyclodextrins are used as ingredients in many different approved medicines, partially due to their hydrophobic interior and hydrophilic exterior, which allows them to form complexes with hydrophobic compounds.[1] These complexes can confer solubility and stability, which have been found to be useful in tailoring drug delivery to targeted body tissues. Additionally, these have found uses in preserving food, where they are commonly used as additives to mask undesired smells.[2]

There are 3 major forms of cyclodextrin, which differ based on their number of glucose subunits: α-cyclodextrin (6 glucose subunits), β-cyclodextrin (7 glucose subunits), and γ-cyclodextrin (8 glucose subunits).[3] Cyclodextrin is formed by the enzymatic breakdown of starch, which is composed of amylose (a linear glucose polymer), and amylopectin (a branched glucose polymer).

Hydroxypropyl betadex, otherwise known as 2-hydroxypropyl β–cyclodextrin, is a form of β–cyclodextrin that has undergone a transformation of some of its OH groups into hydroxypropyl groups by reaction with propylene oxide (**Figure 1**).

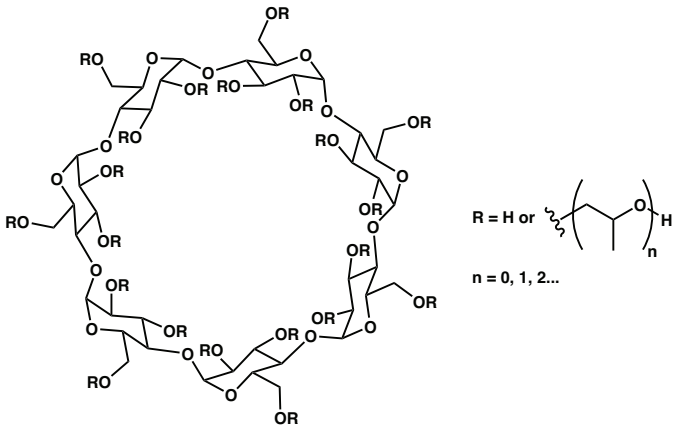

Figure 1. *General structure for β-cyclodextrin (R = H) and hydroxypropyl betadex (R = -(CH$_2$-CH(CH$_3$)-O)$_n$-H).*

Molar substitution test

The molar substitution (MS) of hydroxypropyl betadex is defined as the number of hydroxypropyl groups per anhydroglucose unit. In these USP-NF/Ph. Eu. monograph methods,[4] ^{1}H nuclear magnetic resonance (NMR) is used to calculate the MS by comparing the three protons of the methyl group contained in the hydroxypropyl functional group (A_1) and the signal from the C_1 glycosidic proton (A_2).[5] While the official methods state that analyses must be performed on a spectrometer operating at a ^{1}H frequency of at least 250 MHz, the work herein was performed on a 60 MHz instrument, which shows sufficient performance metrics in both signal-to-noise ratio (SNR) and peak resolution to determine the MS accurately and reliably. To further demonstrate equivalency to the monograph method, the same analyses were repeated on a 400 MHz instrument.

Results

The method was applied to 6 different hydroxypropyl betadex samples, some of which were supplied with known MS values. Specifically, the samples will be referred to as **HPB1380** (MS = 0.6), **HPB1460** (MS = 0.9), **HPBH107** (MS = 0.8), **HPBUSP** (MS = N/A), **HPBW7HP** (MS = 0.6), and **HPBH0979** (MS = N/A). To determine reproducibility, 3 samples for each product were prepared. Additionally, each sample was analyzed in triplicate to confirm repeatability. The results of these studies and comparisons to the label values provided by the supplier (when available) are presented in **Table 1.**[6]

Table 1. *Summary of the average A_2 integration areas at both 60 MHz and 400 MHz, in addition to the calculated MS values.*

Hydroxypropyl Betadex	Sample	A_2[b] 60 MHz	A_2[b] 400 MHz	MS[c] (NMR)
HPB1380 (MS = 0.6)[a]	1	52.6 (0.4)	54.1 (0.5)	0.6
	2	51.8 (0.5)	54.3 (0.2)	0.6
	3	53.8 (0.9)	53.9 (0.8)	0.6
HPB1460 (MS = 0.9)[a]	1	38.3 (0.5)	38.2 (0.6)	0.9
	2	38.0 (0.7)	38.1 (0.8)	0.9
	3	38.6 (1.1)	38.2 (0.4)	0.9
HPBH107 (MS = 0.8)[a]	1	50.6 (0.7)	48.4 (0.5)	0.7
	2	50.3 (1.1)	48.9 (1.1)	0.7
	3	50.9 (0.6)	48.8 (1.6)	0.7
HPBUSP (MS = N/A)[a]	1	58.1 (1.3)	55.8 (0.7)	0.6
	2	57.6 (0.7)	56.0 (1.0)	0.6
	3	58.9 (0.6)	55.8 (0.3)	0.6
HPBW7HP (MS = 0.6)[a]	1	54.0 (0.7)	52.7 (0.7)	0.6
	2	55.0 (0.8)	52.4 (0.6)	0.6
	3	55.1 (1.2)	52.5 (0.1)	0.6
HPBH0979 (MS = N/A)[a]	1	39.1 (0.5)	38.2 (0.7)	0.9
	2	38.9 (0.9)	37.7 (0.8)	0.9
	3	38.7 (1.2)	38.1 (0.4)	0.9

[a]Label value provided by the supplier. [b]Average of triplicate analyses, the relative standard deviation (RSD) values for which are provided in parentheses. [c]MS values determined from both 60 MHz and 400 MHz analyses (these matched for all samples and are presented in a single column). Note: the integration areas for A_1 are not shown here, as these were normalized to 100.0 in all cases.

According to the details provided to us by the suppliers, the MS value for **HPB1380** was determined using an NMR method, while the Ph. Eu. monograph method was specifically used to determine this value for **HPBW7HP**. Details on the methods used to determine these values for **HPB1460** and **HPBH107** were not provided to us. Additionally, the value for **HPBH107** is provided as the degree of substitution (DS = 6), which is obtained by multiplying the MS value by 7, relating to the number of glucose subunits in β-cyclodextrin.

As evidenced by the values obtained in this study, the MS test is well-suited to analysis via benchtop NMR spectroscopy. Overall, great accuracy and precision are observed. While analyses at lower magnetic fields can sometimes give rise to overlap issues due to decreased dispersion, the regions of importance in these samples are well-resolved. In particular, the A_2 region is baseline separated from the residual water signal. Sample preparation is facile, and each analysis takes only ~5 minutes to complete. An example of a ^{1}H NMR spectrum of hydroxypropyl betadex collected for this study is presented in **Figure 2**.

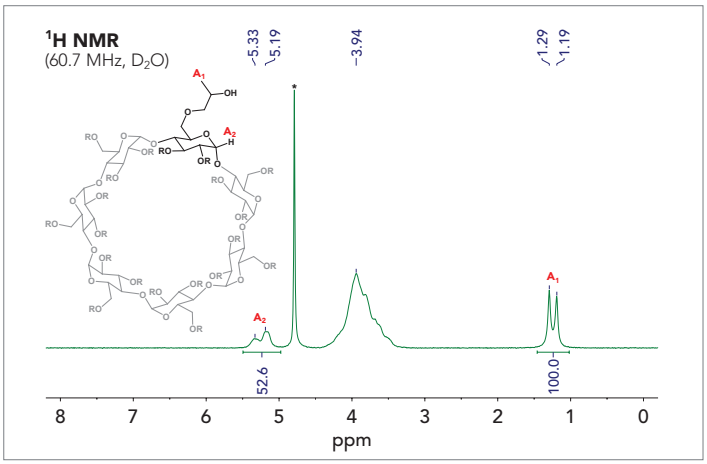

Figure 2. *^{1}H (60.7 MHz) NMR spectrum of **HPB1380** in D_2O with the structure of a representative glucose subunit shown. The protons of the hydroxypropyl methyl group are observed around 1.2 ppm (A_1), while the C_1 glycosidic proton is observed around 5.2 ppm (A_2). The asterisk represents the residual solvent peak for D_2O.*

If you have any questions about the incorporation of benchtop NMR into your USP-NF/Ph. Eur. workflows, or about the work presented herein, please don't hesitate to contact us!

References

(1) Del Valle, E. M. M. *Process Biochem.* **2004**, *39*, 1033–1046.
(2) Davis, M. E.; Brewster, M. E. *Nat. Rev. Drug Discov.* **2004**, *3*, 1023–1035.
(3) Marques, H. M. C. *Flavour Fragr. J.* **2010**, *25*, 313–326.
(4) (a) Hydroxypropyl Betadex (2020). The United States Pharmacopoeia – The National Formulary. Rockville, MD, USA.
(b)Hydroxypropyl Betadex – Monograph 1804 (2019). The European Pharmacopoeia. Strasbourg, France.
(5) The molar substitution can be calculated using the following equation: $MS = A_1/(3 \times A_2)$. Full details can be found in the official monograph methods.
(6) Hydroxypropyl betadex products were received from various suppliers and used without further purification. *MilliporeSigma*: **HPB1380** p/n: 332593, lot: MKCP6785; **HPB1460** p/n: 332607, lot: BCCF2131; **HPBH107** p/n: H107, lot: WXBD4554V. *United States Pharmacopoeia*: **HPBUSP** p/n: 1329709, lot: R144X0. *Ashland*: **HPBW7HP** p/n: 826762, lot: A2102A0118. *Tokyo Chemical Industry (TCI)*: **HPBH0979** p/n: H0979, lot: PZB6E.