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# "Back to the Future": A New Look at Hydroxypropyl Beta-Cyclodextrins

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ABSTRACT

Since the discovery about 30 years ago (2-hydroxypropyl) beta-cyclodextrin, a highly soluble derivative of beta-cyclodextrin, has become an approved excipient of drug formulations included both in the United States and European Pharmacopoeias. It is recommended to use as solubilizer and stabilizer for oral and parenteral formulations. Recently, its pharmacological activity has been recognized in various diseases. The increasing applications require a closer look to the structure-activity relationship. As (2-hydroxypropyl) beta-cyclodextrin (HPBCD) is always a mixture of isomers with various degrees and pattern of hydroxypropylation, no wonder that the products of different manufacturers are often different. Several HPBCDs were compared applying a battery of analytical tools including thin layer chromatography, high performance liquid chromatography (HPLC), HPLC–mass spectrometry (MS), and matrix-assisted laser desorption MS. We studied how the average degree of substitution affects the aggregation behavior, the toxicity, and the solubilizing effect on poorly soluble drugs. We found that the products with low average degree of substitution are more prone to aggregation. The samples studied are nontoxic to Caco-2 cells and have low hemolytic activity. The solubility enhancement of poorly soluble drugs decreases or increases with increasing degree of substitution or shows a maximum curve depending on the properties of the guest.



## Introduction

The use of (2-hydroxypropyl) beta-cyclodextrin (HPBCD) in pharmaceutical formulations was patented almost simultaneously by Janssen Pharmaceutica[1] and National Institutes of Health[2] in 1983 and 1984, respectively. Since then, approximately 3000 articles and 1000 patents on HPBCD have been recorded in the Cyclodextrin News Database of the cyclodextrin-related literature.[3] Called as hydroxypropylbetadex, it became an approved excipient, and a monograph for HPBCD has been published in both the European Pharmacopoeia and US Pharmacopoeia 28/National Formulary 23. It was a rocky road to reach there. Marcus Brewster has played a significant role and left his footprint both in recognizing the properties of HPBCD and proving its safety.[4-7] Further reviews on pharmaceutical application and toxicology of HPBCD have been published by other authors.[8-12]

Nowadays, HPBCD is the most versatile excipient among the cyclic oligosaccharides; it can be used in oral, rectal, dermal, ocular, and parenteral formulations.[13] There are several marketed pharmaceutical products containing HPBCD with various active ingredients.[14] The formulations are mostly solutions (infusion, injection, and eye drops) utilizing the solubilizing and stabilizing effect of HPBCD.

In the novel applications, HPBCD is not an excipient but an active pharmaceutical agent. First, Pitha[15] suggested that HPBCD administered parenterally as a solubilizer of a poorly soluble drug would not remain empty after the release of the drug but might encapsulate lipophilic components, such as vitamins and hormones, within the body. As early as in 1987, his group used intravenous HPBCD treatment for siblings in hypervitaminosis A (unable to metabolize vitamin A) to remove excess vitamin A.[16] This was the very first application of HPBCD as a drug and not as excipient.

Since then, it was discovered that HPBCD has beneficial effects for patients in Niemann Pick type C disease (NPC). This rare,

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genetically inherited disease is a fatal metabolic disorder manifested in enhanced cholesterol accumulation within the brain and other organs resulting in neurodegeneration. HPBCD can slow down the progression of the disease[17] and receive the orphan drug designation for the treatment of NPC from the US Food and Drug Administration[18] and the European Medicinal Agency.[19] Sporadic treatments started in various countries. HPBCD is now in phase 2b/3 clinical trial at the National Institutes of Health and received the US Food and Drug Administration granting of Breakthrough Therapy designation.[20] HPBCD can extract cholesterol from cells with lower capacity than the methylated beta-cyclodextrin (BCD) derivatives,[21] therefore less cytotoxic.[22] It is considered a cholesterol solubilizer, but the mechanism how it helps to reduce the symptoms of NPC disease is not fully understood.

There are several similarities in the pathogenesis of NPC and Alzheimer disease (AD), the most common type of senile dementia.[23] The neuroprotective effect of HPBCD was demonstrated in a transgenic mouse model of AD: spatial learning and memory deficit was highly improved, the beta amyloid level reduced. Age-related macular degeneration is connected with impaired cholesterol efflux in the senescent macrophages in the eyes. HPBCD was patented as intravitreal injection to solubilize the lipids, mostly cholesterol, deposited underneath the retina.[24] Beneficial effects in atherosclerosis were observed in hyperlipidemic rabbits after repeated injections of HPBCD.[25,26] The antiviral effect against HIV,[27] influenza A,[28] and *Herpes simplex*,[29] and so forth as well as the antibacterial effect against *Vibrio cholerae*[30] are based also on cholesterol complexation and with this inhibition of the internalization of both the enveloped viruses[31] and bacteria.[32]

Recently the anticancer effect of HPBCD has been discovered and proved *in vivo* in mouse model of leukemia.[33] The mechanism is again based on modulating the cholesterol homeostasis resulting in inhibition of the growth of leukemic cells. Although all these potential therapeutic effects, except those for NPC treatment, are still in the discovery phase, the standardization of HPBCD might be an urgent need.

The HPBCD samples can be characterized by the degree of substitution (DS, the average number of substituents on a cyclodextrin (CD) molecule). The acceptable range of DS of HPBCD as pharmaceutical excipient is between 2.8 and 10.5 corresponding to 0.4-1.5 substituents per glucose units both in the current European and US Pharmacopoeias. The new potential applications of HPBCD as a drug may require more detailed characterization and strict specification of HPBCD before it will be accepted by the authorities.

The average DS can be determined by various methods, such as nuclear magnetic resonance (NMR), mass spectrometry (MS), reductive-cleavage method and methylation analysis, differential scanning calorimetry,[34] microcalorimetric titration,[35] near infrared reflectance spectroscopy,[36] and colorimetric determination of 1,2-propanediol.[37] The Pharmacopoeias require NMR, but this method provides no information about the isomer distribution.

There are some literature data on the importance of DS. For instance, HPBCD with DS >3 has infinite solubility (even 75% concentration was achieved),[38] but the samples with lower DS (DS <2.5) have poorer solubility than BCD itself.[39] The specific rotation decreased as the DS increased in the range of 2.5-11.3.[40] Similar tendency was obtained for water sorption at 75% relative humidity showing reduced hygroscopicity. The surface activity as well as osmotic pressure gradually increased.[38,41] The hemolyzing effect as well as the complexing ability decreased slightly with increasing DS.[40]

The DS and the distribution of the variously substituted isomers might influence the biological and pharmacological effects. Three HPBCD samples with DS 4.2, 4.3, and 6.7 (from different manufacturers) were compared in the treatment of NPC in mice. The samples with DS 4.2 and 6.7 delayed the onset of neurological symptoms but that with DS 4.3 did not.[42] The large difference in the effect of HPBCDs with approximately the same average DS underlines the importance of the detailed characterization. Various manufacturers produce HPBCDs with various average DS within the acceptable range of 2.8-10.5 confirming the Pharmacopoeia requirements, but that does not guarantee identical efficiency.

In this work, we show the results of an in-depth study on the isomer distribution and substitution patterns of various commercial HPBCDs by analysis of both the intact HPBCDs and the methanolyzed hydroxypropylated glucose units applying sophisticated analytical techniques, which were unavailable at the time of discovery of this important CD derivative. In particular, HPLC with evaporative light scattering detector (HPLC-ELSD) and mass spectrometry detector (HPLC-MS), matrix-assisted laser desorption and ionization time of flight (MALDI-TOF) MS, and 2 dimensional (2D) NMR spectroscopy were used for the characterization of HPBCDs. The methanolyzed samples were analyzed by HPLC-MS with electron spray ionization (ESI)-TOF detector. We introduce a simple HPLC method for representation of component distribution by a fingerprint. The importance of DS was demonstrated by evaluating the solubilizing properties toward selected model compounds as well as aggregation behavior and cytotoxicity data of the commercial HPBCD samples with different average DS.

## Materials and Methods

### *Materials*

The HPBCD samples are products of Wacker Chemie, Germany (DS 4.6 and 6.3), CycloLab, Hungary (DS 3.3 and 9.7), Roquette, France (DS 6.7), and Hangzhou, China (DS 11.1).

The cholesterol and the drugs were purchased from Sigma-Aldrich. As model compounds diclofenac sodium salt and voriconazole, hydrocortisone, cholesterol, tolnaftate, and lovastatin were selected.

The solvents and other chemicals used for the analysis were of analytical grades purchased from Sigma-Aldrich.

Activated carbon, powder, −100 particle size (mesh) was purchased from Sigma.

Aluminum oxide 90 active neutral, activity stage I for column chromatography 0.063-0.200 mm (70-230 mesh American Standard Test Sieve Series) was purchased from Merck.

The thin layer chromatography (TLC) plates were TLC silica gel 60 $F_{254}$ 20 × 20 cm from Merck.

### *Methanolysis Reaction*

All the HPBCD samples were methanolyzed according to the following procedure. The HPBCD sample (3 mmol) was added to methanol (25 mL) under stirring. After complete dissolution, concentrated sulfuric acid (cc $H_2SO_4$) was added (1 mL) and the mixture was stirred under reflux for 2 days (the reaction progress was followed by TLC). The mixture was diluted with methanol (100 mL) and then neutralized with sodium hydroxide (3 N, ~7 mL). The resulting mixture was filtered on a glass sinter, the mother liquor was clarified by activated carbon and then evaporated to dryness. The residue was dissolved in *n*-propanol and chromatographed on neutral aluminum oxide. The fractions were collected and finally evaporated to dryness.

### *TLC of Methanolyzed Samples*

The methanolyzed samples were dissolved in methanol in a 1% concentration (wt/vol), and 2 μL of the solution was spotted on the TLC plate. $CH_3CN$:MeOH:$NH_4OH$ (25%) = 2:1:1 (vol/vol/vol) was