Pharmaceutical Research, Vol. 16, No. 12, 1999

Commentary

Some Important Considerations in the Use of Cyclodextrins

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Received July 7, 1999; accepted August 16, 1999

Cyclodextrins are cyclic oligosaccharides consisting of glucopyranose molecules linked (together) by 1,4 glycosidic bonds. Figure 1 represents an isomer of a hydroxypropyl substituted betadex (β-cyclodextrin), where 2, 3, and 6 represent the three possible reactive sites for adding substituents. In recent years, the use of cyclodextrins in formulation development has increased dramatically. This increase has been driven by the synthesis of derivatives of the parent molecule with improved physicochemical properties, most notably the 2-hydroxypropyl, sulfobutylether, and methylated derivatives of betadex. The physicochemical properties of cyclodextrins, including their ability to form complexes with substrate molecules, may be greatly affected by the type, number and *position* of the substituents on the parent molecule. The distribution and size of the substituents are important to complexation since the primary hydroxyls (C_6) are located around the smaller opening (i.e., the primary face) of the cyclodextrin (CD) truncated cone and the secondary hydroxyls (C_2, C_3) are located around the larger opening, the secondary face (1). Due to the technical difficulty in determining the position of the substituents, the "degree of substitution" is only stated as an "average" where the value given represents the average of the number of substituents on the various glucopyranose groups comprising the CD molecule. Thus, the degree of substitution *per se* does not uniquely characterize a betadex derivative such as hydroxypropyl-β-cyclodextrin (HPBCD) (2). For example, two HPBCD samples produced under different conditions and having the same "degree of substitution" may not have identical physicochemical properties since the hydroxypropyl groups may occupy different positions on the parent molecule. In addition to the "pattern" of 2 versus 3 versus 6 substitution, the distribution of the substituents may vary. Thus, a cyclodextrin with an average degree of substitution of 7 ± 3 may behave differently than one with an average degree of substitution of 7 ± 6. Thus it may be appropriate to develop methods whereby some measure of the distribution of the substitution pattern, e.g., a polydispersity index (3), could be reported.

Despite the importance of the "degree of substitution" there is apparently a considerable amount of confusion surrounding its meaning. In order to discuss this problem some definitions found in the recent literature are an appropriate starting point.

D.S. (the degree of substitution) represents the average number of substituted hydroxyls per glucopyranose unit of the cyclodextrin ring (4). This number represents an average of the analytical determinations and thus, for a given cyclodextrin, it can theoretically be any number between 0 and 3. Since there are 3 reactive hydroxyls per glucopyranose unit, the maximum number of substituents possible are 18, 21, and 24, for α, β and γ-CD, respectively.

M.S. (the average molar degree of substitution) is defined as the average number of moles of the substituting agent, e.g., hydroxypropyl, per mole of glucopyranose (5,6). Unfortunately, this term is often confused with the D.S. The M.S. does not necessarily describe the extent to which the reactive sites are substituted when the substituting agent has reactive sites itself, or when new reactive sites are formed during the substitution reaction. For substituted cyclodextrins this value can be more than 3.0 for each glucopyranose unit, or more than 18, 21, or 24 for α, β, or γ-CD, respectively, since, in the case of HPBCD, the propylene oxide used in the synthesis can react with the hydroxyl group of a hydroxypropyl substituent, forming oligomeric and even polypropylene glycol side chains. Fig. 1 illustrates how the hydroxyl group on the hydroxypropyl substituent can react further with propylene oxide to generate a polymerized side chain(s). Theoretically, there is no upper limit to the M.S. value.

Conversely, when there are no additional reactive sites produced as a result of the substitution reaction, the M.S. and D.S. will be equal. In fact, the M.S./D.S. ratio has been used (4) to indicate the degree of polymerization (**D.P.**) of the side chain(s).

Another way of defining the "degree of substitution" which has appeared in the literature is to describe the substitution in terms of the average number of substituents *per cyclodextrin molecule*. Various terms have been used to describe the "degree of substitution" this way, including M.D.S., the molar degree of substitution (1), the "average degree of substitution" (7), or the "degree of substitution" (8). To avoid the obvious confusion introduced by these terms, we suggest the introduction of a new term, the total degree of substitution (**T.D.S.**), which represents "the average number of substituted groups (e.g., hydroxypropyl) per cyclodextrin molecule." We believe the term "total" is appropriate here since this definition relates to the average number of substitutents on the entire (total) cyclodextrin molecule rather than on one of its repeating (glucopyranose) units. Thus for HPBCD, which has seven glucopyranose units, this value can theoretically range from 0 (for betadex) to > 21.

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0724-8741/99/1200-1796$16.00/0

The preparation of HPBCD involves the reaction of betadex with propylene oxide under alkaline conditions (9). Although, in theory, the nucleophilic attack of the betadex anion can occur at the primary and secondary carbon atoms of propylene oxide, in actual practice the attack occurs almost exclusively at the less sterically-hindered (primary) carbon to produce the 2-hydroxypropyl derivative (10,11). It is important to note here that the 2-HPBCD nomenclature refers to the position of the hydroxyl group on the hydroxypropyl substituents; it does not refer to the reactive site (s) on betadex (i.e., 2, 3, or 6) where substituents can be added. Since the hydroxypropylation reaction involves no net gain or loss of any atoms the molecular formula of HPBCD can be stated as $C_{42}H_{70}O_{35} + (C_3H_6O)_n$, where n represents the number of moles of propylene oxide, i.e., hydroxypropyl substituents, added and therefore

$$MW = 58.08*(T.D.S.) + 1135.00 \quad (1)$$

where: 1135.00 = the MW of betadex ($C_{42}H_{70}O_{35}$), and 58.08 = the MW of propylene oxide (C_3H_6O). Since the β-cyclodextrin molecule consists of 7 glucopyranose units, T.D.S. = 7 *M.S., and thus equation 1 can be restated as

$$MW = 406.56*(M.S.) + 1135.00 \quad (2)$$

Table 1 summarizes these relationships for variously substituted HPBCD molecules. It should be apparent from the above discussion that the MW of the cyclodextrin cannot be calculated using the D.S. when side-chain polymerization occurs, unless the D.P. is known. In the example shown in Figure 1 the D.S. is 4/7 (0.571) and the M.S. is 6/7 (0.857), resulting in a D.P. of 1.5, while the T.D.S. is 6.

Given the relative simplicity of these relationships, what then is the purpose of and justification for this "primer" on cyclodextrin substitution nomenclature? First, and most important, it is apparent from a review of the recent literature on cyclodextrins that these relationships are not uniformly applied. A clear indication of this is that many publications in reputable pharmaceutical journals do not report the D.S., M.S., T.D.S. or the MW of the cyclodextrins being studied. In a survey of some 28 publications on substituted cyclodextrins appearing within the past three years, we observed that only six of these provided sufficient information to enable the reader to determine unequivocally the MW of the cyclodextrin being used. Knowledge of the correct MW of the cyclodextrins enables the reader of a manuscript to compare the reported binding parameters on a mole to mole basis. The lack of this information renders such studies non-reproducible and greatly hinders the interpretation of their findings. One can only conclude that the writers of such manuscripts, their peer-reviewers, and the journal editorial staff do not appreciate the importance of this information in allowing a clear interpretation of the data reported. Second, in many publications the term "degree of substitution" is used throughout the manuscript without the authors ever defining whether this refers to D.S., M.S., or T.D.S (as defined above). For a stated "degree of substitution" of ≤ 3 it is impossible for the reader to determine which of these terms the authors are referring to. In other publications it is apparent that the term "degree of substitution" has been used, on different occasions, to indicate the D.S., M.S. or T.D.S. This terminology problem also extends to the manufacturers and suppliers of cyclodextrins. A comparison of the certificates of analysis from two major cyclodextrin sources (8,12) found that they both used the term "degree of substitution". Unfortunately, in one case this meant M.S. (12) and in the other case it was used to indicate T.D.S. (8), as defined above. Other suppliers merely provide information in their product catalog that the "mean degree of substitution" is "between 4-10" (13). In fact, this supplier provides no information on the container label regarding the "degree of substitution" or the MW of their HPBCD. The researcher must be astute enough to call the supplier and request a certificate of analysis in order to obtain this information. We feel that this procedure leaves too much to chance. This lack of uniform terminology is extremely unfortunate and confusing and yet, to our knowledge, this problem has never been discussed in the literature.

Table 1. Relationship of M.S. and T.D.S. to Molecular Weight for HPBCD

Total degree of substitution (T.D.S.)	Molar degree of substitution (M.S.)	Molecular Weight (MW) (Daltons)
0	0	1135.00
1	0.143 (1/7)	1193.08
2	0.286 (2/7)	1251.16
3	0.429 (3/7)	1309.24
4	0.571 (4/7)	1367.32
5	0.714 (5/7)	1425.40
6	0.857 (6/7)	1483.48
7	1.0 (7/7)[a]	1541.56
8	1.143 (8/7)	1599.64

[a] An M.S. of 1 indicates that there are 7 substituted hydroxyls distributed over the 7 glucopyranose units of the betadex ring structure (7/7), an average of one substituent per glucopyranose unit.

Another problem that needs to be addressed is that different methods of determining the "degree of substitution" can sometimes produce dramatically different values (2). At present the USP is preparing a monograph for HPBCD (14) in which they have recommended the somewhat tedious and unreliable Zeisel reaction (15) to determine the degree of substitution for HPBCD. This is somewhat surprising in view of the fact that newer and more reliable methods, e.g., NMR, FAB-MS (2) and FT-IR (12) have been developed and are currently being used by the manufacturers of HPBCD. Clearly, the definitions for characterizing the "degree of substitution" and the analytical procedures used to determine this parameter are in need of some form of "global harmonization". This can only occur if an increased awareness of the existence of these problems and an appreciation of their importance are achieved. It is our hope that this "primer" will facilitate this process. One recommendation that can be implemented very easily is that all manuscripts reporting on substituted cyclodextrins, and the certificates of analysis provided by manufacturers and suppliers of cyclodextrins, be required to provide the "degree of substitution" and to define which "degree of substitution" they are reporting. Ideally, the certificates of analysis should also state the method used to determine the degree of substitution. Two other problems that relate to the practical use of cyclodextrins in commercial formulations are as follows. First, like many amorphous materials, cyclodextrins are hygroscopic and can pick up moisture when exposed to humidity. Thus, it is important to define the water content of cyclodextrins when preparing samples to achieve a given concentration. Finally, the purity of the cyclodextrins should be

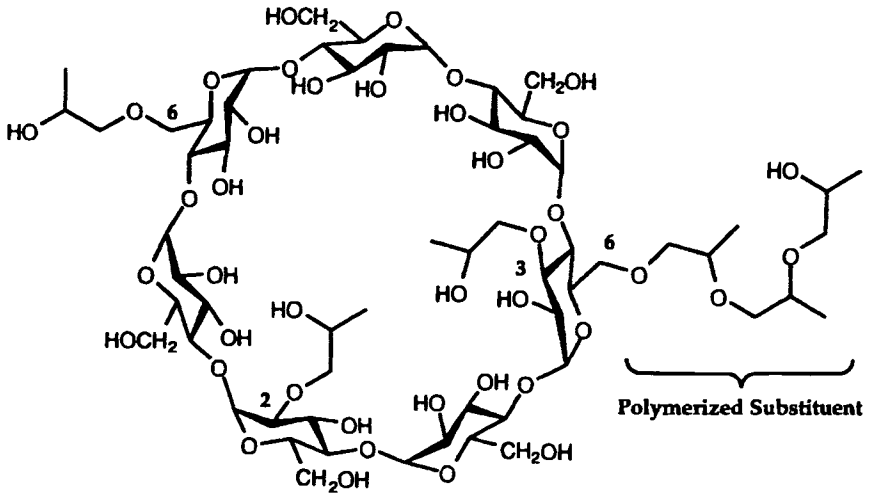

Fig. 1. The chemical structure of one of the isomers of a tetra-substituted hydroxypropyl-β-CD, showing substituents attached at the 2, 3, and 6 positions. The fourth substituent illustrates the further polymerization of the hydroxypropyl substituent with propylene oxide. Permission to use this figure (adapted from reference 1) is acknowledged with thanks to the author, journal and Begell House Publishers, Inc.

known with a high degree of accuracy since "impurities" such as unreacted β-cyclodextrin or pyrogens can have a profound influence on the efficacy and marketability of the finished formulation.

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