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Aspects of determining the molecular weight of cyclodextrin polymers and oligomers by static light scattering

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ABSTRACT

Methodic issues affecting the use of static light scattering to determine the average molecular weight of cyclodextrin poly- and oligomers are discussed. The critical features which enable accurate measurement such as aggregation behavior and segment–segment interaction are elucidated. Static light scattering data supported with globule size and aggregate state analysis (as determined by dynamic light scattering) allowed the aggregate-free state of the samples to be justified and the ideal globular conformation of the macromolecules to be corroborated. These molecular characteristics were demonstrated for uncharged, charged and fluorescent randomly crosslinked water soluble cyclodextrin poly- and oligomers.

1. Introduction

Cyclodextrin (CD) poly- and oligomers[1] are compounds of peculiar structure as their building blocks are functional oligosaccharide units in themselves. In this sense, single cyclodextrin rings from which the macromolecule is built may be regarded as 'monomers'. The functionality of these monomers comes from their torus-shaped cyclic structure (6–8 glucose units are linked by 1,4-α-glycosidic bonds, denoted as αCD; βCD; γCD, respectively) enabling the encapsulation of hydrophobic guest molecules within their cavities. It is important to distinguish between cyclodextrin polymers and cyclodextrin pendant polymers. Random structure cyclodextrin polymers are prepared by chemical crosslinking of native or derivatized CD monomers whilst linear polymers by polymerizing CD-containing monomers. Cyclodextrin pendant polymers, on the other hand, are synthesized by grafting cyclodextrin molecules to a preformed polymer chain. The scope of present paper is restricted to elucidating the molecular characteristics of randomly crosslinked cyclodextrin polymers soluble in water, especially those crosslinked with epichlorohydrin.

The potential pharmaceutical applications of water soluble CD polymers as drug solubilizing excipients have been discussed for more than two decades (Szeman et al., 1987a,b; Fenyvesi, 1988) and is the subject of continuing research (Jug, Kosalec, Maestrelli, & Mura, 2011; Jug, Maestrelli, Bragagni, & Mura, 2010; Martin, Sanchez, Cao, & Rieumont, 2006). It is noteworthy that some cyclodextrin derivatives such as (2-hydroxypropyl)-β-cyclodextrin (Liu et al., 2009) possess biological activity of their own. Pending adequate characterization, we anticipate that exploration of the bioactivity of cyclodextrin polymers will also be subject of future scientific interest. Still, it is noteworthy that a number of publications disclosing the properties or behavior of CD polymers lack any mention of the molecular weight of the substance(s) studied. The average molecular weight of different water soluble CD polymer samples can range widely between 2×10^3 to a few million Daltons (Renard, Deratani, Volet, & Sebille, 1997). The consequences of the differences in molecular weight are well illustrated by an in vivo study where a ~100 kDa CD-based macromolecule was found to have greatly superior antitumor efficacy when compared to a ~40 kDa molecular weight analogue (Cheng, Khin, & Davis, 2004).

Mocanu, Vizitiu, and Carpov (2001) reviewed various features of cyclodextrin polymers and relevant characterization methods were detailed. In this review, only references dealing with gel permeation chromatography (GPC) and viscometry were cited with regard to the determination of the molecular weight. Another relevant reference (Yudiarto, Kashiwabara, Tashiro, & Kokugan, 2001)

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[1] Herein, in case the term 'polymer' stands alone it is understood as also referring to 'oligomers' which has molecular weight less than 50 kDa.

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optimized the polymerization reaction of βCD using epichlorohydrin in the presence of sodium hydroxide under a range of reaction conditions. The water soluble fractions separated by ultrafiltration also had their average molecular weight characterized by GPC. In more recent works use of GPC technique is routinely applied for studying the molecular weight of uncharged, crosslinked CD-polymers (Wintgens & Amiel, 2010; Zhang, Chen, & Diao, 2011; Moya-Ortega et al., 2011).

Li, Xiao, Li, and Zhong (2004) reported their results on the synthesis and characterization of cationic CD-polymers where the determination of molecular weight was also performed by GPC. Calibration was made using βCD against standard pullulan samples. High performance size-exclusion chromatography equipped with a multiangle laser-light scattering and refractive index detectors was found to be adequate to determine the weight-average molecular weights (M_w) of amylopectins (You, Fiedorowicz, & Lim, 1999), amylopectin and amylose (Lee, Han, & Lim, 2009) and CD polymer samples synthesized via crosslinking β-CD with maleic anhydride (Girek, Shin, & Lim, 2000).

Sainz-Rozas, Isasi, & Gonzalez-Gaitano, (2005) studied the hydrodynamic radii of different epichlorohydrin crosslinked βCD polymers by dynamic light scattering. The equivalent molecular mass was obtained based upon the Mark–Houwink–Sakurada equation (Harding, Satelle, & Bloomfield, 1992).

To the best of our knowledge there is no previous work available which characterizes crosslinked cyclodextrin polymers which addresses methodic features of static light scattering when used uncoupled from other techniques. In this work we demonstrate the utility of this method to characterize uncharged, charged and fluorescent cyclodextrin polymer samples as an alternative to GPC technique. The accuracy of GPC results depend on the selection of adequate certified standards which is often a matter of compromise, especially in case of novel materials. To ensure accuracy of the light scattering technique, complementary aggregation state studies were performed using dynamic light scattering.

2. Experimental

2.1. Materials

2.1.1. Neutral cyclodextrin polymer samples

Water soluble neutral CD-polymers were synthesized from α-, β- and γCD by chemical crosslinking in basic media using epichlorohydrin. The obtained products are denoted as PACD, PBCD and PGCD, respectively. The in-detail preparation method is published elsewhere (Fenyvesi, 1988).

2.1.2. Anionic cyclodextrin poly- and oligomers

Carboxymethyl βCD poly- or oligomers (PCMβCD-Na or OCMβCD-Na) may be prepared from carboxymethyl βCD sodium salt (CMβCD-Na) in analogous manner as PBCD. These substances are commercialized products of CycloLab Ltd.

2.1.3. Fluorescent (rhodaminyl) cyclodextrin oligomer sample

OCMβCD-Na was prepared in the following way: CMβCD-Na (DS = 3.7) (9 g, 6.3 mmol) was dissolved in water (8 mL) and the temperature was increased to 60 °C. A solution of NaOH (3 g dissolved in 12 mL) and epichlorohydrin (6 mL) was gradually added. After 2 h stirring at 60 °C the reaction mixture was cooled to room temperature.

OCMβCD-Na was labeled with fluorophore in the same vessel. Rhodamine B isothiocyanate (RBITC, 130 mg, 0.24 mmol) was added and the pink solution was stirred for a further 2 h. The reaction was followed by TLC using 1,4-dioxane:ammonium hydroxide (25%)–10:7 (v/v) as eluent. The reaction mixture was neutralized with HCl 5 N and the unreacted (or decomposed) RBITC was removed by extracting the aqueous phase with dichloromethane till the color of the extract became from dark red to pale pink. The aqueous phase was dialyzed using cellulose membrane (MWCO approx. 1000, from Sigma Aldrich) and freeze drying yielded rhodamine-labeled CMβCD oligomer sodium salt (Rh-OCMβCD-Na) as a pink powder (8.4 g).

The rhodaminyl content in Rh-OCMβCD-Na was estimated by UV–vis spectrophotometry using a calibration curve of rhodaminyl isothiocyanate solutions of 0.03–0.3 mg/L concentrations in MilliQ® water at 260 nm. 0.4 w% rhodaminyl content was obtained.

Fig. 1 represents the structural illustration of the characterized substances.

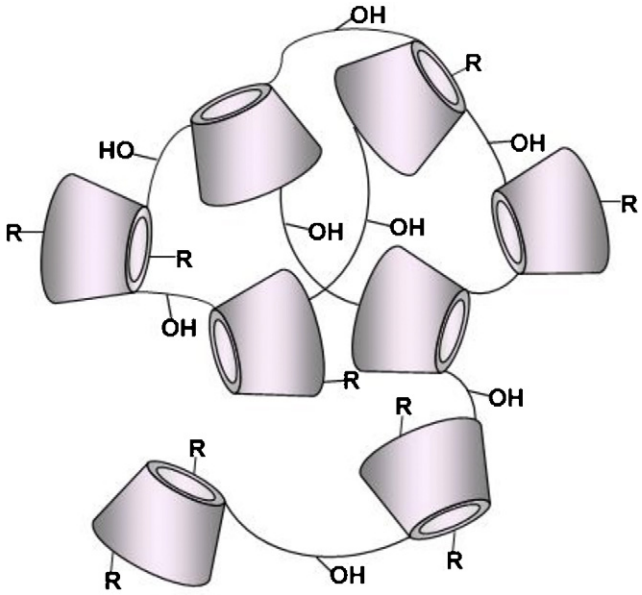

Fig. 1. Schematic structure of crosslinked beta-cyclodextrin oligomers. *R* = —OH: Neutral beta-cyclodextrin oligomer. *R* = —OH or —OCH_2COONa: OCMβCD-Na. *R* = —OH or —OCH_2COONa or rhodaminyl: Rh-OCMβCD-Na.

3. Methods

3.1. Static light scattering

Molecular weight determination was performed using a Malvern Zetasizer Nano ZS instrument running a Static Light Scattering (SLS) method. The samples were studied at different concentrations at 25 °C applying the Rayleigh equation, which describes the intensity of light scattered from the polymer globules in solution:

$$\frac{Kc}{R_\Theta} = \left(\frac{1}{M_w} + 2A_2c\right) P(\Theta),$$

wherein R_Θ, the Rayleigh ratio – the ratio of scattered light to incident light of the sample; M_w, weight-averaged molecular weight; A_2, 2nd virial coefficient; c, concentration; $P(\Theta)$, angular dependence of the sample scattering intensity; K, optical constant as defined as:

$$K = \frac{4\pi^2}{\lambda_0^4 N_A}\left(n_0 \frac{dn}{dc}\right)^2,$$

wherein N_A, Avogadro's constant; λ_0, laser wavelength; n_0, solvent refractive index; dn/dc, the differential refractive index increment.

n_0 and dn/dc were measured using an Abbe refractometer (Karl Zeiss, Jena) at 25 °C. In a case where the polymer globules are