

# Cyclodextrin triggered dimensional changes of polysaccharide nanogel integrated hydrogels at nanometer resolution

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High resolution monitoring of dimensional changes of cholesterol bearing pullulan (CHP) hydrogels was performed as a model for materials' dimensional changes associated with drug delivery. Hydrogels consisting of methacryloyl group-modified cholesterol bearing pullulan (CHPMA) were covalently attached at the end of an optical fibre for interferometric monitoring of dimensional changes. Hydrogels polymerized by CHPMA self-assembled nanogels with different methacryloyl groups (4 and 7 methacryloyl groups per 100 glucose units) at different original CHP concentrations (35, 45 and 55 mg mL<sup>-1</sup>) were employed to prepare soft materials with various swelling properties. The substituted cholesteryl groups in CHP gels affect the hydrogel swelling by forming association domains by hydrophobic interaction that can also be destabilized by host–guest interactions with cyclodextrins. The swelling properties were determined with 2 nm resolution in optical length and sampled at a frequency of approximately 1 Hz for the 50  $\mu$ m radius hemispherical hydrogels. The results show that the equilibrium swelling and swelling kinetics of the CHP depend on its composition and the exposure to cyclodextrin types and concentrations. CHP hydrogels with the least number of methacryloyl groups and the lowest CHP concentration yielded the largest swelling changes on exposure to methyl- $\beta$ -cyclodextrin. The swelling rate induced by cyclodextrin was independent of CHP concentration and type of cyclodextrin. The interferometric investigation of CHP hydrogel swelling associated with the disassociation of cholesterol group aggregates has proved its potential in providing information on hydrogel swelling relevant to materials' dimensional changes associated with controlled drug delivery.

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## 1 Introduction

Research focusing on optimal design of biocompatible systems for controllable drug release over a predefined period of time is of prime interest for controlled drug delivery.<sup>1</sup> Hydrogels, a cross-linked polymer network that can swell in water, realized using various molecular building blocks, have been extensively explored for their applicability in the development of a controlled drug delivery system.<sup>2,3</sup> Nanogels are defined as aqueous dispersions of hydrogel particles (<100 nm) with three-dimensional networks of cross-linked polymer chains. Nanogels have attracted much attention due to their unique

physicochemical properties and applications in biomaterials.<sup>4–6</sup> Several types of nanogels whose physical or chemical properties (e.g., swelling ratio) can respond to external stimuli, such as temperature,<sup>7,8</sup> pH,<sup>9–11</sup> light,<sup>12–14</sup> and the presence of molecules,<sup>15,16</sup> have been studied. Notably, Akiyoshi *et al.* have developed physically cross-linked nanogels which are composed of polysaccharides as main chains and grafted hydrophobic molecules such as cholesterol (~5 mol%).<sup>17</sup> For instance, cholesterol-bearing pullulan (CHP) forms nanogels (20–30 nm) in water by self-assembly. The main driving force of the nanogel formation is hydrophobic interaction *via* cholesteryl groups as functions of association domains.<sup>17</sup>

The cholesterol association domains of the nanogel possess unique properties and specific functions for the nanogel, including the ability to encapsulate hydrophobic drugs or soluble proteins.<sup>18,19</sup> The destabilization of association domains could be expected to control or tune the properties and functions of CHP nanogels. Cyclodextrin (CD) has the ability to act as a suitable host for cholesteryl groups due to its hydrophobic cavity.<sup>20–22</sup> Cyclodextrin stimulated dissociation of CHP nanogels, acting by forming host–guest complexes with the

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cholesteryl groups and thereby suppressing the hydrophobically driven cholesterol self-assembly, has been reported.<sup>23</sup> For these unique properties, CHP nanogels act as artificial molecular chaperones where proteins were trapped inside the nanogel matrix to inhibit the aggregation and released as native forms by addition of CD.<sup>24,25</sup> Furthermore, CHP hydrogels, especially the covalently cross-linked CHP hydrogel, demonstrate nanogel formation inside the gel matrix and retain chaperone-like activity to trap and release protein by host-guest interactions of the cholesteryl group and CD. This is a new hydrogel consisting of self-assembled nanogels as building blocks. The nanogel-based hydrogels are useful in a sustained protein delivery system for tissue engineering.<sup>26,27</sup> To understand the physico-chemical properties of the CHP nanogel (hydrogel) from molecular to macroscopic level in detail, the structural change detection at the nanometer scale needs to be investigated. However, the relative studies investigated by dynamic light scattering (DLS) or small-angle neutron scattering could not illustrate the roles of CHP composition and its aggregate states in the interaction between CHP nanogels and cyclodextrin.<sup>28–30</sup>

An interferometric readout platform has recently provided high resolution data for determination of swelling response and swelling kinetics of various responsive hydrogels.<sup>31</sup> The responsive hydrogel is in this tool deposited at the end of an optical fiber, and the analysis of the reflected, net interference wave from the reflections at the optical fiber-hydrogel and hydrogel-immersion solution, respectively, yields information on the optical length within the hydrogel material. This tool has been applied to monitoring the ionic strength and pH response of ionic hydrogels,<sup>32</sup> carbohydrates in particular glucose induced swelling of phenylboronic acid functionalized hydrogels,<sup>33,34</sup> ssDNA induced swelling changes and swelling dynamic of DNA hybrid hydrogels,<sup>35–37</sup> swelling response and kinetics of anionic hydrogels associated with polymer impregnation<sup>38,39</sup> and within a hydrogel-surfactant-cyclodextrin system.<sup>40</sup> These

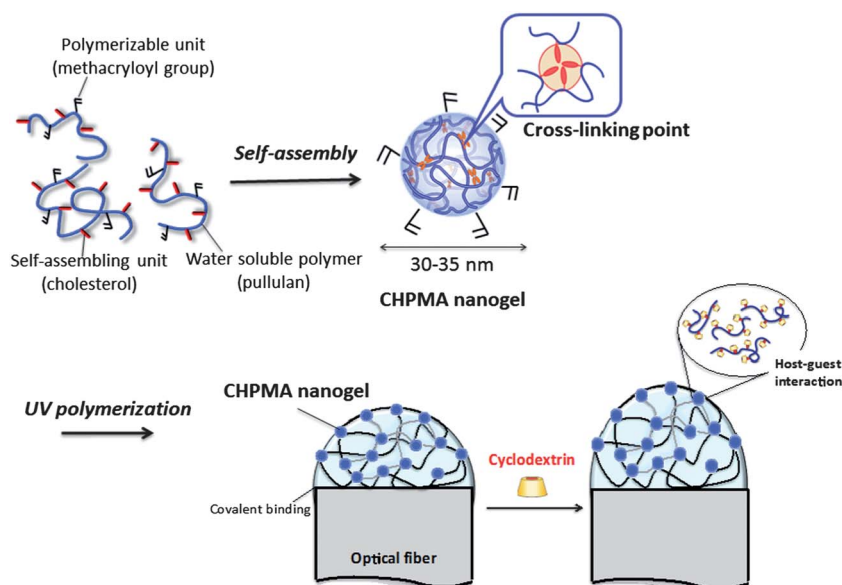
hydrogels attached on the tip of optical fiber were determined with respect to changes in optical length with a resolution of 2 nm and a sampling frequency of about 1 Hz.

The aim of the present work is to provide more details of swelling response of CHP hydrogels on exposure to cyclodextrins as compared to characterization of CHP nanogels by scattering techniques. Towards this end, we utilize the interferometric technique to investigate the swelling equilibrium ratio and the rate of CHP gels on exposure to cyclodextrins (Fig. 1). We used polymerizable group modified CHP and prepared hemispherical CHP microgels (radius of about 50  $\mu\text{m}$ ) at the end of the optical fiber to detect the swelling response. The relative factors for hydrogel equilibrium swelling are considered as (1) the type of cyclodextrins included based on their different interaction strengths with cholesterol, (2) the substitution ratio of reactive groups mediating cross-linking by UV polymerization of methacryloyl group modified CHP (CHPMA), and (3) the concentration of the CHPMA in the pregel solution. Such characterization aids towards a molecular understanding of the changes in materials behavior upon stimulated release, and as such considered as relevant models in materials development for controlled drug delivery.

## 2 Experimental

### 2.1 Materials

CHP, in which pullulan ( $M_w = 1.0 \times 10^5 \text{ g mol}^{-1}$ ) was substituted with 1.2 cholesteryl groups per 100 glucose units, was a gift from Nippon Oil and Fat Co. (Tokyo, Japan). Methacryloyl group modified CHP (CHPMA) was synthesized as reported previously<sup>41</sup> and its chemical structure is shown in Fig. 2. The degree of substitution (DS) of methacryloyl groups was 4 (CHPMA4) and 7 (CHPMA7) per 100 glucose units, as determined by  $^1\text{H}$  NMR.<sup>41</sup> Ethanol (VWR, 96%), 3-(trimethoxysilyl)propyl methacrylate (Sigma, >98%) and hydrochloric acid



**Fig. 1** Schematic description of the preparation and the swelling changes induced by cyclodextrin of cholesterol bearing pullulan (CHP) hydrogels.

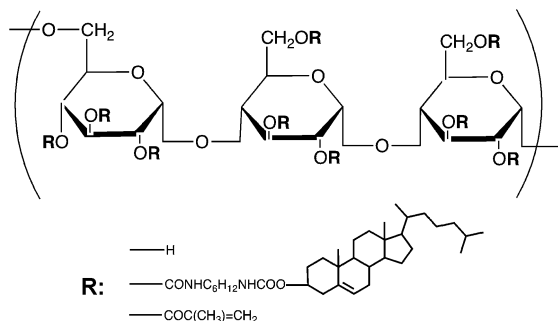


Fig. 2 Chemical structure of methacryloyl group modified CHP.

(37%, Merck) were used in the methacrylate functionalization process at the end of the optical fibers.  $\alpha$ -Cyclodextrin ( $\alpha$ -CD, 98%, Fluka),  $\beta$ -cyclodextrin ( $\beta$ -CD, 97%, SAFC), methyl- $\beta$ -cyclodextrin (m- $\beta$ -CD, 97%, Aldrich) and  $\gamma$ -cyclodextrin ( $\gamma$ -CD, 99%, Aldrich) were used as received for determination of swelling properties of the hydrogels under various conditions. Photoinitiator (hydroxy-cyclohexyl phenyl ketone, 99%, Aldrich) added in squalane (2,6,10,15,19,23-hexamethyl tetracosane, 99%, Aldrich), with the concentration of 2.6 mg ml<sup>-1</sup>, was used to provide the polymerization environment. Deionized water (resistivity 18.2 M $\Omega$   $\times$  cm, obtained using a Millipore setup) was used for all aqueous solutions.

## 2.2 Preparation of CHP hydrogels on optical fiber

The optical fiber was stripped of the jacket, cleaned, and cut before being activated and functionalized with methacrylate for covalent grafting of the hydrogels onto the end of the optical fiber.<sup>32</sup> The methacrylate groups covalently attached to the end of the fiber ensure covalent binding of the polymer network molecules. An appropriate amount of CHPMA was dissolved in the deionized water (45 °C) for 6 h to yield 35, 45 or 55 mg ml<sup>-1</sup> pregel solution. The 45 mg ml<sup>-1</sup> CHP concentration corresponds to 3 mM of cholesterol. Aliquots of the CHPMA nanogel solution were deposited at the end of the functionalized fiber immersed in a droplet of squalane as previously outlined.<sup>32</sup> The pregel solutions were polymerized under UV light (Dymax Bluewave 50 equipped with a light guide) for 45 min. The hemispherical CHPMA microgels were subsequently washed with deionized water for at least one day to remove possible unpolymerized nanogels and other impurities.

## 2.3 Interferometric characterization of hydrogel swelling

All experiments were carried out at room temperature with the hydrogels immersed in the aqueous solution under constant agitation using a magnetic stirrer. The hydrogels covalently linked to the end of the optical fibers were protected against mechanical damage during exposure to changing solvent by locating them within glass tubes with an inner diameter of about 5 mm. The hydrogels were equilibrated by immersion in 8 ml of deionized water (equilibrium indicated by fluctuations in the phase signal of the interferometer less than the resolution limit). Aliquots of concentrated cyclodextrin solutions were

pipetted to the immersing aqueous solution. The interferometric readout platform provides information on changes of the optical length,  $\Delta l_{\text{opt}}$ , of the hydrogels both in the intensity and phase change of the reflected interference wave. The estimate of  $\Delta l_{\text{opt}}$  was obtained based on the experimentally determined change in phase  $\Delta\phi$  of the interference wave:

$$\Delta l_{\text{opt}} = \frac{\Delta\phi\lambda_0}{4\pi} \quad (1)$$

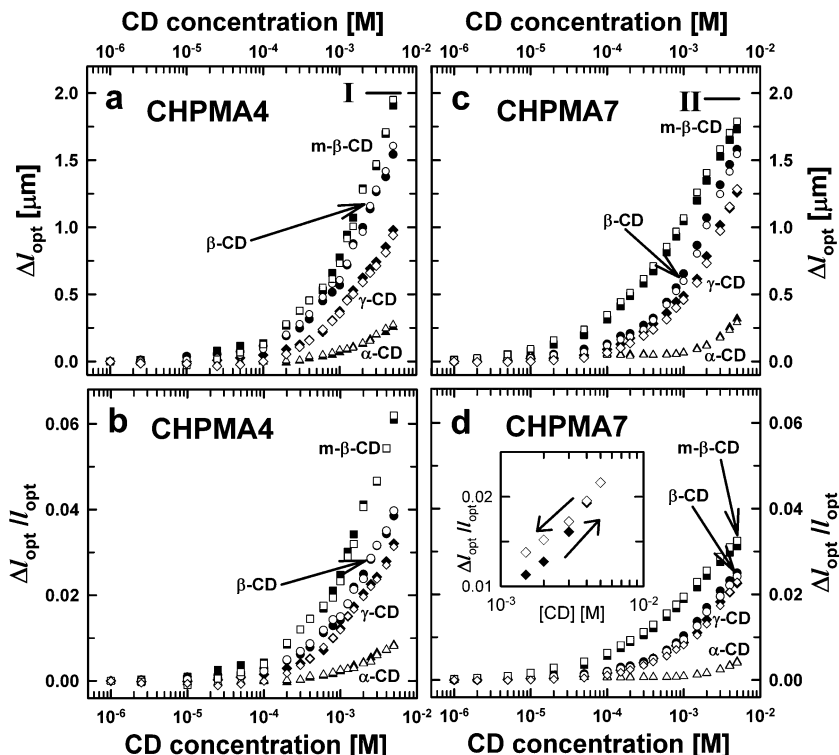
where  $\lambda_0$  is the center wavelength of the light source (1550 nm) because this provides superior resolution compared to data based on the intensity.<sup>32</sup> Parameter  $\Delta l_{\text{opt}}$  was sampled at 1 Hz, and it is shown that this approach has a resolution in  $\Delta l_{\text{opt}}$  of 2 nm. Both parameter  $\Delta l_{\text{opt}}$  relative to a reference state with a given  $l_{\text{opt}}$  for the actual hydrogel and the normalized values  $\Delta l_{\text{opt}}/l_{\text{opt}}$  are reported. The swelling responses of the hydrogels to the stepwise increase of the cyclodextrin concentrations were followed as a function of time and both kinetic curves and equilibrium swelling data are reported.

## 3 Results and discussion

CHP hydrogels display equilibrium swelling changes on exposure to cyclodextrin that depend on the type of cyclodextrin and network parameters, in particular the cross-link density. Hemispherical CHP microgels which were covalently linked to the end of the optical fiber were prepared. To compare the cross-linked density of the CHP hydrogels, two different methacryloyl group contents per 100 glucose units of CHPMA (CHPMA4 and CHPMA7) were used. Their swelling behaviors responding to different types of  $\alpha$ -CD,  $\beta$ -CD, m- $\beta$ -CD and  $\gamma$ -CD were investigated by the interferometric technique.

### 3.1 Swelling responses with different cyclodextrins, and cross-linked density of CHP hydrogels

Fig. 3 displays the difference in the optical length on exposure to cyclodextrins, and this difference is related to the optical length of the reference state of CHP hydrogels with two different contents of methacryloyl groups supporting covalent cross-links CHPMA4 (Fig. 3a and b) and CHPMA7 (Fig. 3c and d). Such data were recorded for a cyclodextrin concentration in the range up to 5 mM. The nearly coinciding data in the two series of data collected for the exposure of each hydrogel to cyclodextrins show the good reproducibility in the swelling behavior (Fig. 3). The CHP hydrogels display a monotonous increase in optical length (swelling) with increasing cyclodextrin concentration from 0 to 5 mM that depends on the type of cyclodextrin and larger swelling response for the less covalently cross-linked CHP hydrogels. The CD sensitivity sequence for the CHP gels is m- $\beta$ -CD,  $\beta$ -CD,  $\gamma$ -CD and  $\alpha$ -CD, which is consistent with the known conclusion that m- $\beta$ -CD was found to be more efficient than  $\beta$ -cyclodextrin to remove cholesterol, and  $\alpha$ -CD was the poorest one.<sup>42</sup> The total change in optical length of CHPMA4 gel exposure to m- $\beta$ -CD for [CD] up to 5 mM is 2  $\mu$ m (Fig. 3a) corresponding to about 6% in the change ratio (Fig. 3b). This is larger than the increase of  $\Delta l_{\text{opt}}$  of 0.25  $\mu$ m, equal to a 1% swelling change ratio, for the CHPMA4 induced by  $\alpha$ -CD.



**Fig. 3** Changes in optical length and swelling ratio for CHP hydrogels with 1.2 cholesterol and 4 (CHPMA4 hydrogels with optical lengths of 33.7  $\mu\text{m}$ , 39.6  $\mu\text{m}$ , 31.5  $\mu\text{m}$  and 30.6  $\mu\text{m}$  respectively for  $\alpha$ -CD,  $\beta$ -CD, m- $\beta$ -CD and  $\gamma$ -CD in (a) and (b)) or 7 (CHPMA7 hydrogels with optical lengths of 72.9  $\mu\text{m}$ , 63.6  $\mu\text{m}$ , 55.1  $\mu\text{m}$  and 55.9  $\mu\text{m}$  respectively for  $\alpha$ -CD,  $\beta$ -CD, m- $\beta$ -CD and  $\gamma$ -CD in (c) and (d)) methacryloyl groups per 100 glucose units in response to a gradual increase of different types of cyclodextrin concentrations. The CHP hydrogels were prepared with the original CHP concentration of 45  $\text{mg ml}^{-1}$ . The measurements were carried out at least two times (open and filled symbols for each experimental series) at room temperature. The regions I and II in the figure depict cyclodextrin concentration ranges selected for the display of time-dependence of  $\Delta l_{\text{opt}}$  (Fig. 5 and 6). Cyclodextrin induced deswelling (open symbols) at decreasing  $\gamma$ -CD concentration from 5 mM ((d), inset).

Increasing the covalent cross-link density, while using pul-lulans with the same substitution ratio of cholesterol in the hydrogels, yields hydrogels that display smaller changes in the  $\Delta l_{\text{opt}}$  on exposure to cyclodextrins while preserving CD differences. The effect of methacryloyl substitution ratio on cyclodextrin induced swelling response of CHP hydrogels was observed from CHP hydrogels with the substitution ratio of 4 and 7 methacryloyl groups per 100 glucose units and the same original CHP concentration of 45  $\text{mg ml}^{-1}$ . The relative swelling increase at 5 mM CD of the CHPMA4 hydrogels was 6%, 4%, 3.3%, and 1%, with a monotonous increase in swelling in the range of [m- $\beta$ -CD], [ $\beta$ -CD], [ $\gamma$ -CD] and [ $\alpha$ -CD], respectively. These relative swelling ratios decreased to 3.3%, 2.5%, 2.3% and 0.5% for the CHPMA7 hydrogels at 5 mM of the various CDs (Fig. 3b and d). The deswelling of the CHP hydrogel was found to possess a substantial degree of reversibility ( $\gamma$ -CD, Fig. 3d, inset), but was not completely reversible for equilibration times of 1000 s at each level of [ $\gamma$ -CD].

Analysis of the difference in cyclodextrin induced changes in the swelling of the CHP hydrogels to estimate the relative binding constants of the different CDs is carried out by the extract relative CHP hydrogel volume changes from the primary observable  $\Delta l_{\text{opt}}$ , followed by consideration of the effect of the various cyclodextrins on the swelling. The previous analysis strategy<sup>40</sup> of decomposing the primary observable  $\Delta l_{\text{opt}}$  to changes due to altered optical properties of the hydrogels and change in the physical length,

$$\Delta l_{\text{opt}} = \langle n_2 \rangle l_2 - \langle n_1 \rangle l_1 \approx \langle n_1 \rangle \Delta l + l_1 \Delta n \quad (2)$$

is implemented. In eqn (2), indices 1 and 2 represent the two states to be compared, *e.g.* with and without CD for the CHP hydrogels, and

$$\langle n_i \rangle = l_i^{-1} \int_0^{l_i} n_i(l) dl, \quad i = 1, 2 \quad (3)$$

are the average of the refractive indices along the optical pathway of the two states. The refractive index of the CHP hydrogels at 45  $\text{mg ml}^{-1}$  is estimated to be 1.37 employing the reported refractive index increment  $dn/dc = 0.150 \text{ ml g}^{-1}$  for 1.2 mol% cholesterol substituted pullan.<sup>43</sup> The contribution  $l_1 \Delta n / l_{\text{opt}}$  to  $\Delta l_{\text{opt}} / l_{\text{opt}}$  due to the presence of cyclodextrin is estimated to be less than  $4 \times 10^{-4}$  at a CD concentration of 3 mM, using a  $dn/dc$  of  $0.148 \text{ ml g}^{-1}$ .<sup>44</sup> This contrasts most of the experimentally determined relative changes  $\Delta l_{\text{opt}} / l_{\text{opt}}$  being typically 100 times or larger. Thus, the term  $\langle n_1 \rangle \Delta l$ , *i.e.*, the changes in physical length of the hydrogels, represent the dominating contribution to the experimentally determined  $\Delta l_{\text{opt}}$  changes.

The relative binding constants of the various CDs are estimated assuming the same relative volume changes, *e.g.*,  $V/V_0 = 1.02$ , representing equal perturbation of the cholesterol domains by the CD. The experimentally determined  $\Delta l_{\text{opt}} / l_{\text{opt}}$  is converted to  $V/V_0$  using the relationship,  $V/V_0 \sim [(\Delta l_{\text{opt}} + l_{\text{opt}}) / l_{\text{opt}}]^{2.6}$  (Fig. 4),



obtained from finite element analysis of swelling of hydrogels constrained at the fiber optical base.<sup>45</sup> The power law coefficient of 2.6 different from 3 arises due to constraining of the hydrogel to the end of the optical fiber resulting in an increase of the length of the hydrogel along the fiber axis and decrease of the overall swelling volume relative to unconstrained swelling. Selecting  $V/V_0 = 1.02$  as the basis for obtaining estimates of CD concentrations that represent equal perturbation of the CHP hydrogels  $[CD]_{ref}$  (Table 1) yields a basis for estimating relative binding constants. The finding that the swelling data collapse to one master curve when presented as a function of  $[CD]/[CD]_{ref}$  (Fig. 4b) indicates that selection of the extent of swelling (value of  $V/V_0$ ) for estimation of  $[CD]_{ref}$  does not influence the ratios of the  $[CD]_{ref}$  for different types of CD.

The data in Table 1 are used as a basis for estimating the relative association constants by assuming that 1 : 2 (cholesterol : CD) are the prevailing molar complexes since these are formed more easily than 1 : 1 molar complexes.<sup>46</sup> The lack of a clear plateau in the swelling data *versus* the CD concentrations can also reflect that the 1 : 2 cholesterol : CD complexes are the

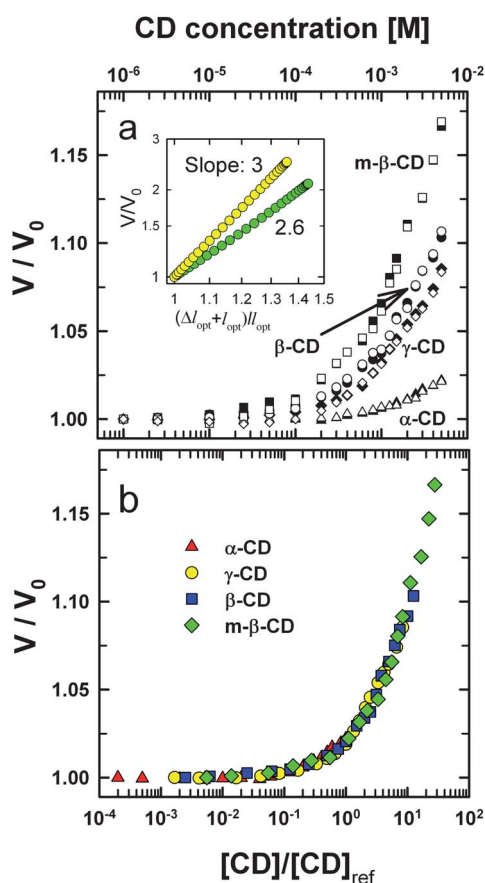
**Table 1** Experimentally determined cyclodextrin concentrations inducing swelling of CHP hydrogels to  $V/V_0 = 1.02$

Hydrogel composition	m- $\beta$ -CD	$\beta$ -CD	$\gamma$ -CD	$\alpha$ -CD
CHPMA4	$0.18 \times 10^{-3}$	$0.40 \times 10^{-3}$	$0.6 \times 10^{-3}$	$5 \times 10^{-3}$
CHPMA7	$0.17 \times 10^{-3}$	$0.7 \times 10^{-3}$	$0.9 \times 10^{-3}$	$>5 \times 10^{-3}$

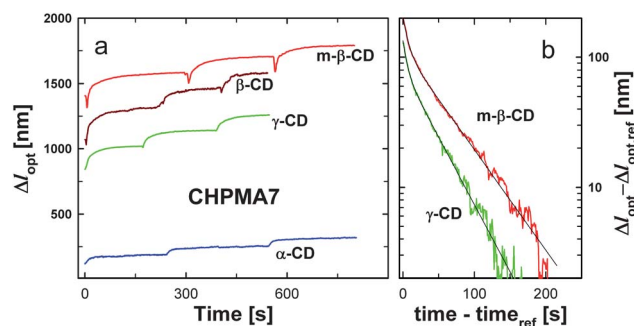
preferred interaction modes. The ratios between the cholesterol : CD association constants are estimated to be 1 : 50 : 100 : 800 for  $\alpha$ -CD,  $\gamma$ -CD,  $\beta$ -CD and m- $\beta$ -CD (relative uncertainties of about 20%). These estimates follow the same trends in the reported capability of solubilizing cholesterol by various cyclodextrins.<sup>42</sup>

### 3.2 Swelling kinetics of CHP hydrogels

Fig. 5 presents the swelling kinetics of the CHPMA7 gels for the step CD concentration changes from 2 to 3, 4 and 5 mM (region II in Fig. 3). No apparent difference in swelling kinetics was observed for the CHP gels exposure to different CDs. The fit of double exponential function to the readjustment to new equilibrium (*e.g.* Fig. 5b) indicated that the data could be accounted for by two apparent time constants of 4–8 s, and about 40–70 s. The apparent time constant in the order of 4–8 s is close to that reported for swelling kinetics of hydrogels of a similar size of 2–3 seconds observed for ionic strength induced swelling change.<sup>32</sup> Time constants in this range most likely reflect the size-dependent hydrogel swelling kinetics. The time constant of 40–70 seconds observed can be compared to apparent equilibration times for other rate limiting processes for the hydrogel swelling. This includes time constants of 200–300 seconds for ionic hydrogels exposed to stepwise changes in pH to larger than 10 min for oligonucleotide-acrylamide hybrid hydrogels exposed to ssDNA complementary to that in the hydrogel.<sup>35,37</sup> The swelling kinetics of ionic hydrogels exposed to the



**Fig. 4** Relative changes in the hydrogel volume  $V/V_0$  of CHP hydrogels with 1.2 cholesterol and 4 methacryloyl groups *versus* cyclodextrin concentrations for  $\alpha$ -CD,  $\beta$ -CD, m- $\beta$ -CD and  $\gamma$ -CD (a) and normalized cyclodextrin concentrations (b). The changes in the optical lengths (Fig. 3a) were converted to relative volume changes using the relationship between the relative volume and physical length along the optical axis determined by finite element analysis of constrained hydrogels at the optical axis ((a), inset, slope = 2.6 on a double logarithmic scale, the data indicated with slope 3 correspond to free swelling).



**Fig. 5** Swelling kinetics of CHPMA7 hydrogels (7 methacryloyl groups per 100 glucose units) with the original CHP concentration of  $45 \text{ mg ml}^{-1}$  in different types of cyclodextrin. The data were obtained from changes in optical length *versus* time for stepwise increase in cyclodextrin concentrations from 2 to 5 mM (region II in Fig. 3c) (a). The swelling state of the CHPMA7 hydrogels with no added cyclodextrin to the aqueous solution was selected as the reference state. (b) Fit of experimental data (red, green) to a double exponential model (black) for the middlemost time course in (a). The reference point for this analysis is selected as the start time, and plateau for each [CD].

surfactant was observed to be in the range 4–300 seconds depending on the concentration range of the surfactant relative to their critical micelle and critical aggregate concentrations.<sup>40</sup>

In this latter series, exposing the surfactant equilibrated hydrogels to cyclodextrins yielded an equilibration time in the order of a few seconds.<sup>40</sup> The equilibration times observed in the present series are much longer than for CD exposure to surfactant equilibrated hydrogels. This could originate from differences in kinetics of the formation of inclusion complexes of cyclodextrins with cholesterol as compared to the surfactants (DTAB and CTAB). A further facet includes possible constraints from the network due to the fact that the cholesterol nanogel domain was allowed to be formed prior to the cross-linking process. Additionally, cyclodextrin destabilization of the cholesterol domains will propagate its swelling response through network chain relaxation directly since the cholesterol groups are grafted onto the network chain. The presence of different CDs was shown to only influence swelling capacity due to the interaction capability between cholesterol and cyclodextrin but not affect swelling kinetics of CHP hydrogels. The data obtained for an apparent hysteresis in the CHP hydrogels on decreasing CD concentrations obtained for 1000 s equilibration at each CD concentration (Fig. 3d, inset) suggest that equilibration associated with re-establishing cholesterol association domains is slower than their CD induced dissociation.

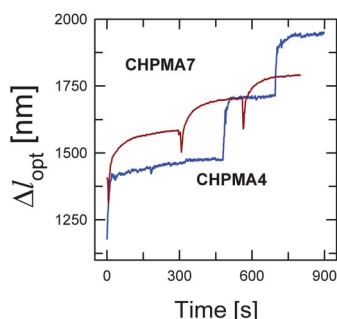
CHP gels modified with methacryloyl groups denote the copolymerization functionality during the UV polymerization. The effect of methacryloyl substitution ratio on cyclodextrin induced swelling response of CHP hydrogels was compared between CHP hydrogels with the substitution ratio of 4 and 7 methacryloyl groups per 100 glucose units and the same original CHP concentration of 45 mg ml<sup>-1</sup>. The swelling change ratios for CHPMA4 hydrogels are 6%, 4%, 3.3%, and 1% with continuous swelling in the whole [m- $\beta$ -CD], [ $\beta$ -CD], [ $\gamma$ -CD] and [ $\alpha$ -CD] ranges corresponding to only 3.3%, 2.5%, 2.3% and 0.5% for CHPMA7 (Fig. 3b and d). However, the swelling kinetics observed was around 300 s for both CHPMA4 and CHPMA7

responding to a stepwise increase of m- $\beta$ -CD concentration in the range from 2 to 5 mM (Fig. 6, selected from regions I and II in Fig. 3a and c).

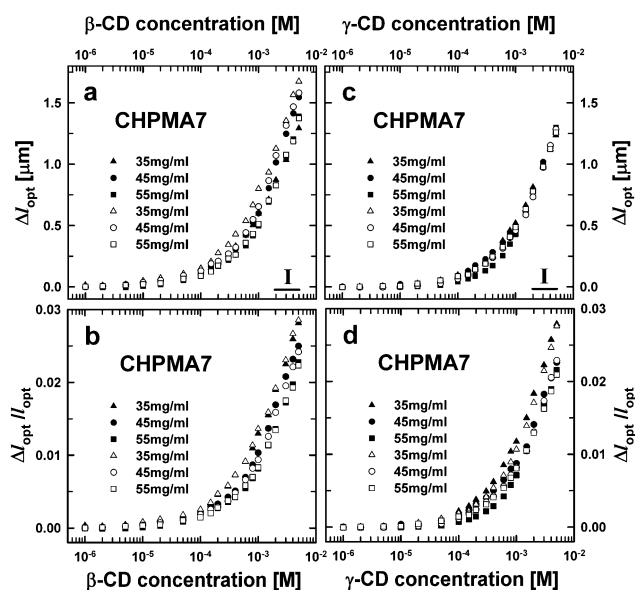
A higher substitution ratio leads to the higher cross-link density. Increasing the cross-link density of hydrogels will reduce the free volume within the hydrogel network structure and lead to the reduction of the water holding capacity. And high cross-link degrees also block the chain relaxation and affect the swelling equilibrium.

### 3.3 Influence of CHP concentration on the gel swelling

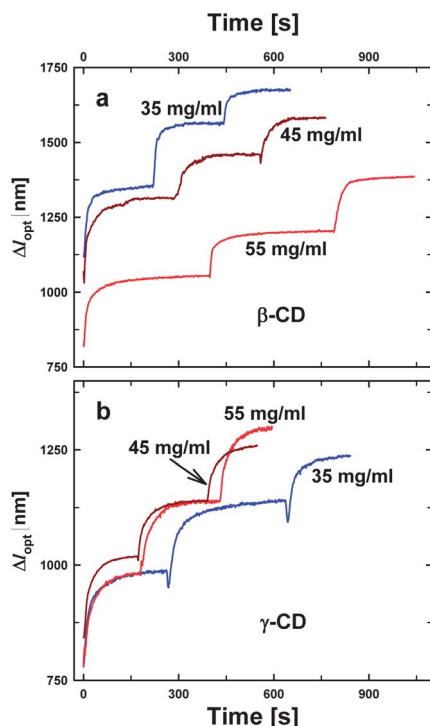
The effect of CHP concentration on the swelling properties of CHP hydrogels was also studied using an interferometer. Fig. 7 displays swelling changes of CHPMA7 hydrogels with different concentrations of 35, 45 and 55 mg ml<sup>-1</sup> on exposure to  $\beta$ -CD (Fig. 7a and b) or  $\gamma$ -CD (Fig. 7c and d). The CHP gels with different concentrations swell on exposure to  $\beta$ -CD. The highest swelling change ratio in the whole  $\beta$ -CD concentration was observed for CHPMA7 gels with the lowest concentration of 35 mg ml<sup>-1</sup> despite only slight difference shown. The same condition was observed for CHPMA7 gels on exposure to  $\gamma$ -CD. A low concentration of CHP gels leads to high swelling changes on exposure to CDs. Fig. 8 displays selected swelling kinetics of CHPMA7 hydrogels in response to a stepwise increase of  $\beta$ -CD (Fig. 8a) or  $\gamma$ -CD (Fig. 8b) in the concentration ranges: 1–5 mM (respectively indicated in Fig. 7a and c). No apparent difference in swelling kinetics can be found in CHPMA7 gels with different CHP concentrations on exposure to  $\beta$ -CD, even for  $\gamma$ -CD. The kinetics of CHPMA7 hydrogel response to cyclodextrins was



**Fig. 6** Swelling kinetics of CHP hydrogels with 4 (CHPMA4) and 7 (CHPMA7) methacryloyl groups per 100 glucose units in the original CHP concentration of 45 mg ml<sup>-1</sup> on exposure to m- $\beta$ -CD. The data were obtained from changes in optical length versus time for stepwise increase in m- $\beta$ -CD concentrations from 2 to 5 mM (regions I and II in Fig. 3a and c). The swelling state of the CHPMA4 and CHPMA7 hydrogels with no added cyclodextrins to the aqueous solution was selected as the reference state.



**Fig. 7** Changes in optical length and swelling ratio for CHP hydrogels with 1.2 cholesterol and 7 (CHPMA7) methacryloyl groups per 100 glucose units with different original CHP concentrations of 35 (45.8  $\mu$ m), 45 (55.1  $\mu$ m), and 55 (60.6  $\mu$ m), mg ml<sup>-1</sup> in response to a gradual increase of  $\beta$ -CD (a and b) and  $\gamma$ -CD (c and d) concentrations. The measurements were carried out at least two times (open and filled symbols for each experimental series) at room temperature. The regions I in (a) and (c) respectively depict  $\beta$ -CD and  $\gamma$ -CD concentration ranges selected for the display of time-dependence of  $\Delta L_{\text{opt}}$  (Fig. 8).



**Fig. 8** Swelling kinetics of CHPMA7 (7 methacryloyl groups per 100 glucose units) hydrogels in different original CHP concentrations of 35, 45 and 55 mg ml<sup>-1</sup> on exposure to  $\beta$ -CD (a) and  $\gamma$ -CD (b). The data were obtained from changes in optical length versus time for stepwise increase in CD concentrations from 2 to 5 mM (region I in Fig. 7a and c). The swelling state of the CHPMA7 hydrogels with no added cyclodextrin to the aqueous solution was selected as the reference state.

found to only be limited by the diffusion/reorganization of hydrophobic aggregates of cholesteryl groups but not affected by aggregate states of CHP nanogels.

## 4 Conclusions

In this work we investigate the equilibrium swelling ratio and swelling kinetics of cholesterol bearing pullulan (CHP) hydrogels associated with destabilization of the formed cholesterol hydrophobic association domains in the CHP gels with different types of cyclodextrins ( $\alpha$ -CD,  $\beta$ -CD, methyl- $\beta$ -CD and  $\gamma$ -CD). The CHP gels with different substitution ratios of methacryloyl groups and different original CHP concentrations were allowed for monitoring the swelling behaviors exposed to CD solution by using an optical interferometric technique. The results show continuous swelling properties of CHP gels on exposure to different types of CDs due to the host-guest interaction between cholesterol and cyclodextrin. Methyl- $\beta$ -CD induced the largest swelling changes and  $\alpha$ -CD leads to the smallest swelling changes in the same CHP gels, arising from the capability of supermolecular self-assembly. And the equilibrium swelling ratio in CD solution depends on the degree of substitution of the methacryloyl group and original CHP concentration, which supports the low cross-link degree and loose aggregate state. The swelling kinetics of destabilization of hydrophobic interaction inside the CHP hydrogels was shown

to be a factor limiting the rate of hydrogel swelling. The quick swelling rate cannot be reached by different types of CD and original CHP concentration but can only be obtained from CHP gels with a smaller number of methacryloyl groups. These results show high potential in tuning the equilibrium sensitivity ratio and response rate for CHP hydrogels for the development of a controlled drug delivery system.

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