Soft Matter

Dynamic Article Links ►

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

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

Ming Gao[1#], Sayaka Toita[2,3#], Shin-ichi Sawada[2,3], Kazunari Akiyoshi[2,3] and Bjørn Torger Stokke[1]*

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

High resolution monitoring of dimensional changes of cholesterol bearing pullulan (CHP) hydrogel was performed as a model for materials dimensional changes associated with drug delivery. Hydrogels consisting of methacryloyl groups modified cholesterol bearing pullulan (CHPMA) were covalently attached to 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) in different original CHP concentrations (35, 45 and 55 mg/ml) 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 also can be destabilized by host- guest interaction with cyclodextrins. The swelling properties were determined with 2 nm resolution in optical length and sampled at frequency of approximately 1 Hz for the 50 μm radius hemispherical hydrogels. The results show that the equilibrium swelling and swelling kinetics of the CHP depend on their composition and the exposure to cyclodextrins type and concentrations. Hydrogels with the lowest methacryloyl groups and lowest CHP concentration yielded the largest swelling changes on exposure to methyl-β-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 of hydrogel swelling relevant of materials dimensional changes associated with controlled drug delivery.

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.[1] Hydrogels, a crosslinked polymer network that can swell in water, realized using various molecular building blocks, have been extensively explored for their applicability in the development of controlled drug delivery system.[2, 3] 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.[4-6] Several types of nanogels whose physical or chemical properties (*e.g.*, swelling ratio) can respond to external stimuli, such as temperature,[7, 8] pH,[9-11] light,[12-14] and the presence of molecules,[15, 16] have been studied. Notably, Akiyoshi *et al.* have developed physically cross-linked nanogels which are composed of polysaccharides as main chain and grafted hydrophobic molecules such as cholesterol (~5 mol%).[17] For instance, cholesterol-bearing pullulan (CHP) form nanogel (20-30 nm) in water by self-assembly. The main driving force of the nanogel formation is hydrophobic interaction via cholesteryl groups as functions on association domains.[17]

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.[18, 19] 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.[20-22] Cyclodextrin stimulated dissociation of CHP nanogels, acting by forming host-guest complexes with the cholesteryl groups and thereby suppressing the hydrophobically driven cholesterol self-assembly, have been reported.[23] For these unique properties, CHP nanogel acts as an artificial molecular chaperone where proteins were trapped inside the nanogel matrix to inhibit the aggregation and released as native forms by addition of CD.[24, 25] Furthermore, CHP hydrogel, especially covalently cross-linked CHP hydrogel, possess nanogel formation inside the gel matrix and retained chaperone-like activity to trap and release protein by host-guest interaction of the cholesteryl group and CD. This is a new hydrogel consisting of self-assembled nanogel as a building block. The nanogel-based hydrogels are useful in sustained protein delivery system for tissue engineering.[26, 27] To understand the physicochemical properties of CHP nanogel (hydrogel) from molecular to macroscopic level in detail, the structural changes

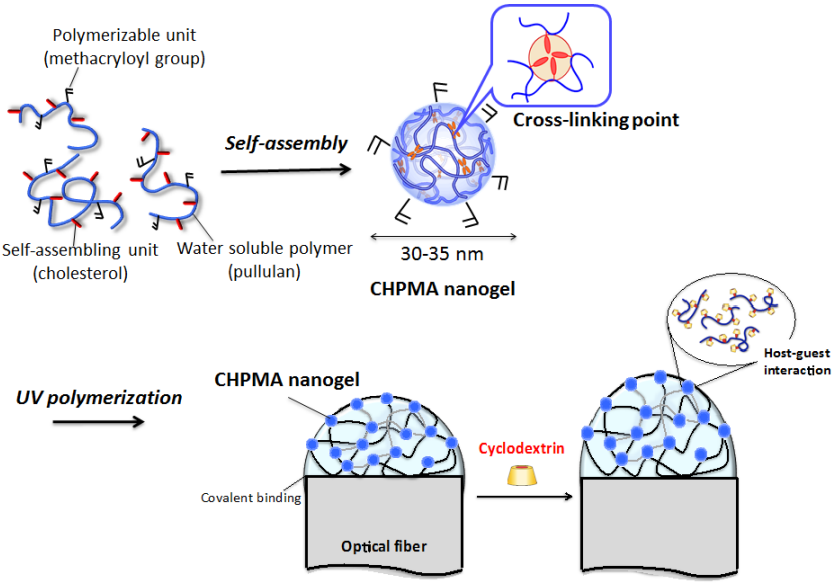

Figure 1. Schematic description of the preparation and the swelling changes induced by cyclodextrin of cholesterol bearing pullulan (CHP) hydrogels.

detection at the nanometer scale need 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.[28-30]

An interferometric readout platform has recently provided high resolution data in determining swelling response and swelling kinetics of various responsive hydrogels.[31] The responsive hydrogel is in this tool deposited at the end of an optical fiber, and analysis of the reflected, net interference wave from the reflections at the optical fiber - hydrogel and hydrogel - immersion solution, respectively, yields information of the optical length within the hydrogel material. This tool has been applied in monitoring the ionic strength and pH response of ionic hydrogels,[32] carbohydrates in particular glucose induced swelling of phenylboronic acid functionalized hydrogels,[33, 34] ssDNA induced swelling changes and swelling dynamic of DNA hybrid hydrogels,[35-37] swelling response and kinetics of anionic hydrogels associated with polymer impregnation[38, 39] and within hydrogel- surfactant- cyclodextrin system.[40] These hydrogels attached on the tip of optical fiber were determined with respect to changes in optical length with 2 nm resolution and sampling frequency at 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 rate of CHP gels on exposure to the cyclodextrins (Figure 1). We used polymerizable group modified CHP and prepared hemispherical CHP micro gels (radius of about 50 μm) 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 cyclodextrin included based on their different interaction strengths with cholesterol, 2) the substitution ratio of reactive groups mediating crosslinking by UV polymerization of methacryloyl group modified CHP (CHPMA), and 3) 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 × 10^5 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[41] and its chemical structure was shown in Figure 2. The degree of substitution (DS) of methacryloyl groups were 4 (CHPMA4), and 7 (CHPMA7) per 100 glucose units, determined by ^{1}H-NMR.[41] Ethanol (VWR, 96%), 3-(trimethoxysilyl)propyl methacrylate (Sigma, >98%) and hydrochloric acid (37%, Merck) were used in the methacrylate functionalization process at the end of the optical fibers. α-cyclodextrin (α-CD, 98%, Fluka), β-cyclodextrin (β-CD, 97%, SAFC), methyl-β-cyclodextrin (m-β-CD, 97%, Aldrich) and γ-cyclodextrin (γ-CD, 99%, Aldrich) were used as received for determination of swelling properties of the

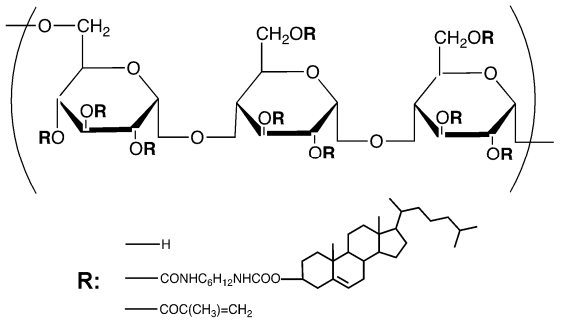

Figure 2. Chemical structure of methacryloyl groups modified CHP

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, was used to provide the polymerization environment. Deionized water (resistivity 18.2 MΩ ×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 the hydrogels to the end of the optical fiber.[32] The methacrylate groups covalently attached to the end of the fiber ensures covalent binding of the polymer network molecules. The appropriate amount of CHPMA was dissolved in the deionized water (45°C) for 6h to yield 35, 45 or 55 mg/ml pregel solution. The 45 mg/ml CHP concentration correspond 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.[32] The pregel solutions were polymerized under UV light (Dymax Bluewave 50 equipped with a light guide) for 45 min. The hemispherical CHPMA micro gels 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 a 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 phase signal of the interferometer less than the resolution limit). Aliquots of concentrated cyclodextrins solutions were pipetted to the immersing aqueous solution. The interferometric readout platform provides information on changes of the optical length, Δl_{opt} of the hydrogels both in the intensity and phase change of the reflected interference wave. The estimate of Δl_{opt} was obtained based on the experimentally determined change in phase $\Delta\varphi$ of the interference wave:

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

where λ_0 is the center wavelength of the light source (1550 nm) because this provides superior resolution compared to data based on the intensity.[32] Parameter Δl_{opt} was sampled at 1 Hz, and it is shown that this approach has a resolution in Δl_{opt} of 2 nm. Both parameter Δl_{opt} relative a reference state with a give l_{opt} for the actual hydrogel, and the normalized values $\Delta l_{opt}/l_{opt}$ are reported. The swelling responses of the hydrogels to the stepwise increase of the cyclodextrins concentration were followed as 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 crosslink 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 α-CD, β-CD, m-β-CD and γ-CD were investigated by the interferometric technique.

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

Figure 3 displays the difference in the optical length on exposure to cyclodextrins, and this difference relative to the optical length of the reference state of CHP hydrogels with two different contents of methacryloyl groups supporting covalent crosslinks CHPMA4 (Figs. 3a and 3b) and CHPMA7 (Figs. 3c, 3d). Such data were recorded for 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 the cyclodextrins show the good reproducibility in the swelling behavior (Fig. 3). The CHP hydrogels display monotonous increase in optical length (swelling) with increasing cyclodextrin concentration from 0 to 5 mM that depended on the type of cyclodextrin and larger swelling response for the less covalently crosslinked CHP hydrogels. The CD sensitivity sequence for the CHP gels is m-β-CD, β-CD, γ-CD and α-CD, which is consistent with the known conclusion that m-β-CD was found to be more efficient than β-cyclodextrin to remove cholesterol and α-CD was the poorest one.[42] The total changes in optical length of CHPMA4 gels exposure to m-β-CD for [CD] up to 5 mM is 2 μm (Fig. 3a) corresponding about 6% in change ratio (Fig.3b). This is larger than the increase of Δl_{opt} of 0.25 μm, equal to 1% swelling change ratio, for the CHPMA4 induced by α-CD.

Increasing the covalent crosslink density, while using pullulans with the same substitution ratio of cholesterol in the hydrogels, yields hydrogels that display smaller changes in the Δl_{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

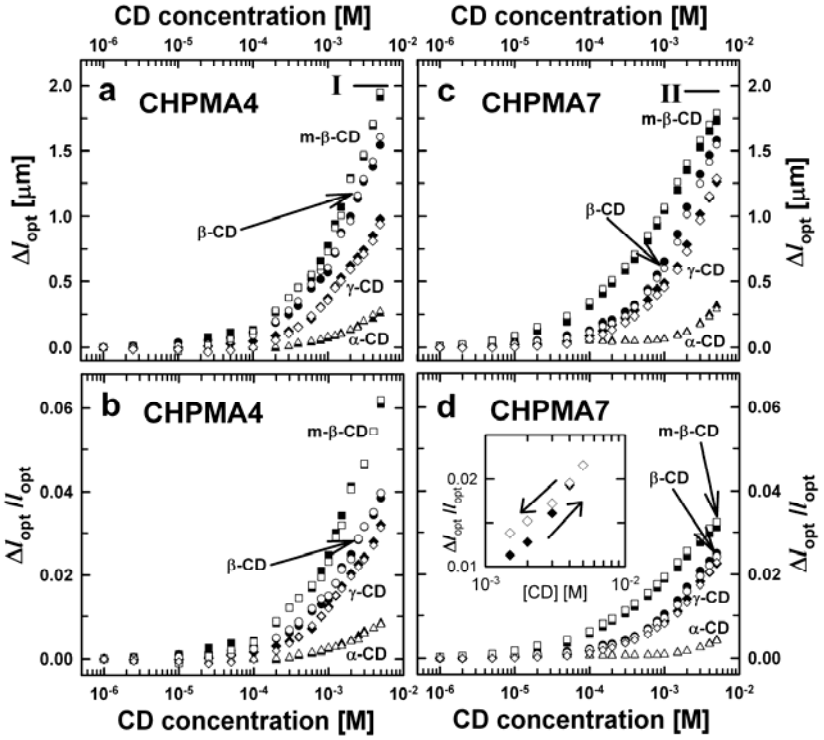

Figure 3. Changes in optical length and swelling ratio for CHP hydrogels with 1.2 cholesterol and 4 (CHPMA4 hydrogels with optical length of 33.7 μm, 39.6 μm, 31.5 μm and 30.6 μm respectively for a-CD, β-CD, m-β-CD and γ-CD in Figure 3a and 3b) or 7 (CHPMA7 hydrogels with optical length of 72.9 μm, 63.6 μm, 55.1 μm and 55.9 μm respectively for a-CD, β-CD, m-β-CD and γ-CD in Figure 3c and 3d) methacryloyl groups per 100 glucose units on response to gradually increased different types of cyclodextrin concentrations. The CHP hydrogels were prepared with the original CHP concentration of 45mg/ml. The measurements were carried out at least two times (open and filled symbols for each experimental series) at room temperature. The region I and II in Figure 3 depict cyclodextrin concentration ranges selected for the display of time-dependence of Δl_{opt} (Figure 5 and Figure 6). Cyclodextrin induced deswelling (open symbols) at decreasing γ-CD concentration from 5 mM (Figure 3d, inset).

concentration of 45 mg/ml. The relative swelling increase at 5 mM CD of the CHPMA4 hydrogels were 6%, 4%, 3.3%, and 1%, with monotonous increase in swelling in the range of [m-β-CD], [β-CD], [γ-CD] and [α-CD]. 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 3d). The deswelling of the CHP hydrogel was found to posess substantial degree of reversibility (γ-CD, Fig. 3d, inset), but was not completely reversible for equilibration times of 1000 s at each level of [γ-CD].

Analyses of the difference in cyclodextrin induced changes in the swelling of the CHP hydrogels to estimate relative binding constants of the different CD is carried out by extract relative CHP hydrogel volume changes from the primary observable Δl_{opt}, followed by consideration of effect of the various cyclodextrins on the swelling. The previous analysis strategy[40] of decomposing the primary observable Δl_{opt} to changes due to altered optical properties of the hydrogels and change in the physical length,

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

are implemented. In eq. 2, indeces 1 and 2 represent the two states to be compared, e.g. with and without CD for the CHP hydrogels, and

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

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

The relative binding constants of the various CDs are estimated assuming the same relative volume changes, e.g., $V/V_0 = 1.02$, represent equal perturbation of the cholesterol domains by the CD. The experimentally determined $\Delta l_{opt}/l_{opt}$ is converted to V/V_0 using the relation $V/V_0 \sim [(\Delta l_{opt}+l_{opt})/l_{opt}]^{2.6}$ (Fig. 4) obtained from finite element analysis of swelling of hydrogels constrained at the fiber optical base.[45] 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 collapses to one master curve when presented as function of $[CD]/[CD]_{ref}$ (Fig. 4b) indicate that selection of extent of swelling (value of V/V_0) for estimation of $[CD]_{ref}$ does not influence the ratios of the $[CD]_{ref}$ for the different types of CD.

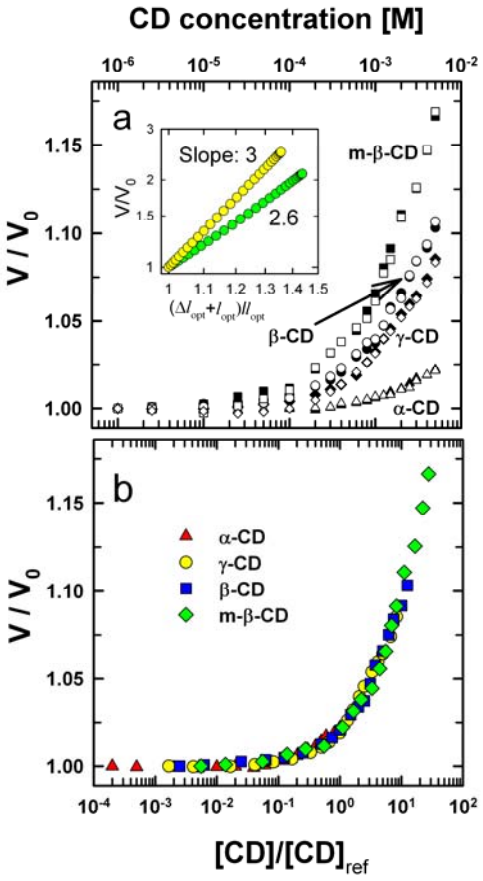

Figure 4. Relative changes in hydrogel volume V/V_0 of CHP hydrogels with 1.2 cholesterol and 4 methacryloyl groups versus cyclodextrin concentrations for α-CD, β-CD, m-β-CD and γ-CD (a) and normalized cyclodextrin concentrations (b). The changes in the optical lengths (Fig. 3a) were converted to relative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

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

Hydrogel composition	m-β-CD	β-CD	γ-CD	α-CD
CHPMA4	$0.18\ 10^{-3}$	$0.40\ 10^{-3}$	$0.6\ 10^{-3}$	$5\ 10^{-3}$
CHPMA7	$0.17\ 10^{-3}$	$0.7\ 10^{-3}$	$0.9\ 10^{-3}$	$>5\ 10^{-3}$

The data in Table 1 are used as a basis for estimating relative association constants assuming that 1:2 (cholesterol:CD) are the prevailing molar complexes since these are formed more easily than 1:1 molar complexes.[46] 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 preferred interaction modes. The ratio between the cholesterol: CD association constants are estimated to 1 : 50: 100 : 800 for α-CD, γ-CD, β-CD and m-β-CD (relative uncertainties of about 20%). These estimates follow the same trends in the reported capability of solubilizing cholesterol by various cyclodextrins.[42]

3.2 Swelling kinetics of CHP hydrogel

Figure 5 presents swelling kinetics for the CHPMA7 gels for the step CD concentration changes from 2 to 3, 4 and 5 mM (region II in Figure 3). No apparent difference in swelling kinetics was observed for the CHP gels exposure to different CDs. Fit of double expontential function to the readjustment to new equilibrium (e.g. Fig. 5b) indicated that the data could be acounted 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 similar size of 2-3 seconds observed for ionic strength induced swelling change. Timeconstants in this range most likely reflect the size-dependent hydrogel swelling kinetics. The timeconstant 40-70 seconds observed can be compared to apparent equilibration times for other rate limiting process for the hydrogel swelling. This include timeconstants of 200-300 seconds for ionic hydrogels exposed to stepwise changes in pH to larger than 10 mins for oligonucleotide-acrylamide hybrid hydrogels exposed to ssDNA complementary to that in the hydrogel.[35, 37] The swelling kinetics of ionic hydrogels exposed to 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.[40]

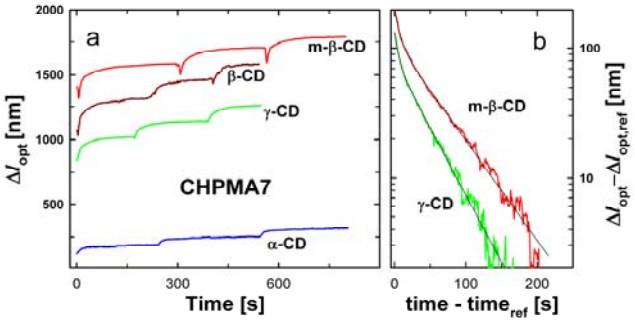

Figure 5. Swelling kinetics of CHPMA7 hydrogels (7 methacryloyl groups per 100 glucose units) with the original CHP concentration of 45 mg/ml in different types of cyclodextrin. The data were obtained from changes in optical length versus time for stepwise increase in cyclodextrin concentrations from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

In this latter series, exposing the surfactant equilibrated hydrogels

to cyclodextrins yielded an equilibration time in the order of a few seconds.[40] 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 include possible constraints from the network due to the fact that the cholesterol nanogel domain were allowed to be formed prior to the crosslinking process. Additionally, cyclodextrin destabilization of the cholesterol domains will propagate its swelling response through network chain relaxation directly than since the cholesterol groups are grafted on 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) suggests that equilibration associated with re-establishing cholesterol association domains are 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 acryloyl per 100 glucose units and the same original CHP concentration of 45 mg/ml. The swelling changes ratio for CHPMA4 hydrogels are 6%, 4%, 3.3%, and 1% with continuous swelling in the whole [m-β-CD], [β-CD], [γ-CD] and [α-CD] ranges corresponding that they are only 3.3%, 2.5%, 2.3% and 0.5% for CHPMA7 (Fig. 3b and 3d). However, the swelling kinetics observed was around 300s for both CHPMA4 and CHPMA7 responding to stepwise increase of m-β-CD concentration in the range from 2 to 5 mM (Figure 6, selected from regions I and II in Figure 3a and 3c).

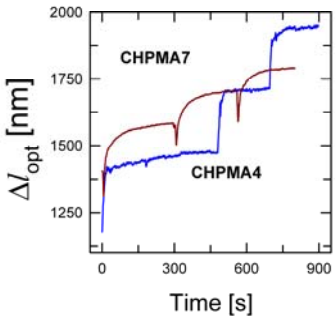

Figure 6. Swelling kinetics of CHP hydrogels with 4 (CHPMA4), 7 (CHPMA7), methacryloyl groups per 100 glucose units in the original CHP concentration of 45mg/ml on exposure to m-β-CD. The data were obtained from changes in optical length versus time for stepwise increase in m-β-CD concentrations from 2 to 5 mM (region I and II in Figure 3a and 3c). The swelling state2 of the CHPMA4 and CHPMA7 hydrogels with no added cyclodextrins to the aqueous solution were selected as the reference states.

Higher substitution ratio leads to the higher crosslink density. Increasing the crosslink 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 crosslink degrees also block the chains 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 by interferometer. Figure 7 displays swelling changes of CHPMA7 hydrogels with different concentration of 35, 45 and 55 mg/ml on exposure to β-CD (Figure 7a and 7b) or γ-CD (Figure 7c and 7d). The CHP gels with different concentration swell on exposure to β-CD. The highest swelling change ratio in the whole β-CD concentration was observed for CHPMA7 gels with lowest concentration of 35 mg/ml despite of only slight difference shown. It is the same condition for CHPMA7 gels on exposure to γ-CD. Low concentration of CHP gels leads to high swelling changes on exposure to CDs. Figure 8 displays selected swelling kinetics of CHPMA7 hydrogels in response to stepwise increase of β-CD (Figure 8a) or γ-CD (Figure 8b) in the concentration ranges: I 2-5 mM (respectively indicated in Figure 7a and Figure 7c). No apparent difference in swelling kinetics can be found in CHPMA7 gels with different CHP concentration on exposure to β-CD, even for γ-CD. The kinetics of CHPMA7 hydrogels response to cyclodextrins was found to only be limited by the diffusion/ reorganization of hydrophobic aggregates of cholesteryl groups but not affected by aggregates states of CHP nanogels

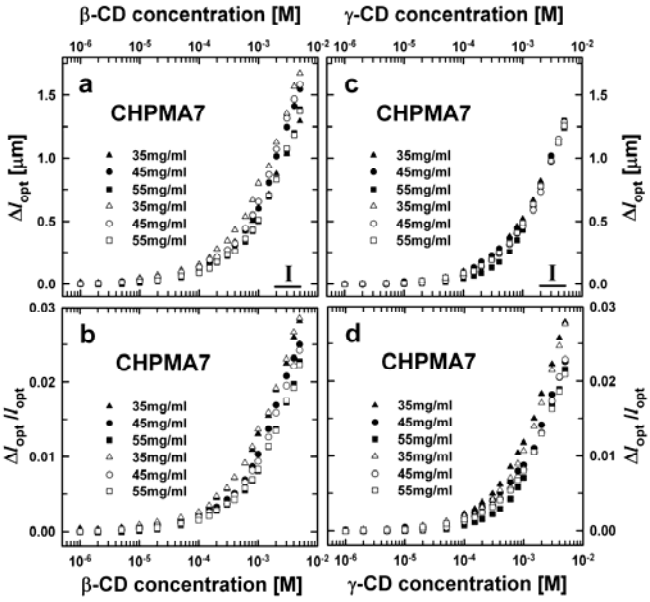

Figure 7. Changes in optical length and swelling ratio for CHP hydrogels with 1.2 cholesterol and 7 (CHPMA7) methacryloyl groups per 100 glucose units with different original CHP concentration of 35 (45.8 μm), 45 (55.1 μm), and 55 (60.6 μm), mg/ml on response to gradually increased β-CD (Figure 7a and 7b) and γ-CD (Figure 7c and 7d) 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 Figure 7a and 7c respectively depict β-CD and γ-CD concentration ranges selected for the display of time-dependence of Δl_{opt} (Figure 8).

.

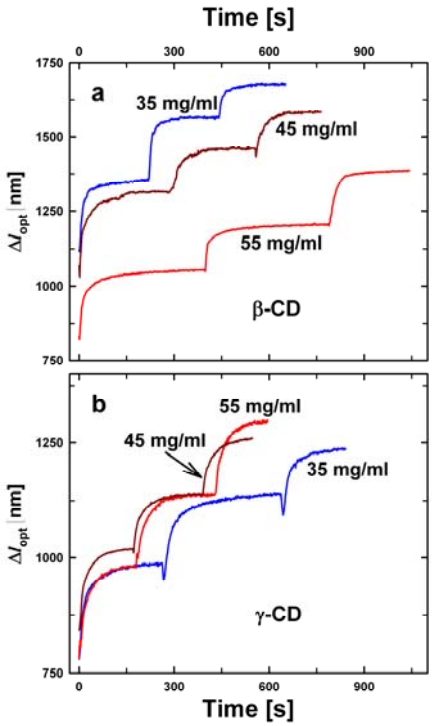

Figure 8. Swelling kinetics of CHPMA7 (7 methacryloyl groups per 100 glucose units) hydrogels in different original CHP concentration of 35, 45 and 55mg/ml on exposure to β-CD (Figure 8a) and γ-CD (Figure 8b). The data were obtained from changes in optical length versus time for stepwise increase in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

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 (α-CD, β-CD, methyl-β-CD and γ-CD). The CHP gels with different substitution ratio of methacryloyl groups and different original CHP concentration were allowed for monitoring the swelling behaviors exposed to CDs solution by using 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-β-CD induced the largest swelling changes and α-CD lead 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 depended on the degree of substitution of methacryloyl group and original CHP concentration, which supporting the low crosslink 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 can not be reached by different CD types and original CHP concentration but only obtained from CHP gels with low methacryloyl groups. These results show high potential in tuning the equilibrium sensitivity ratio and response rate for CHP hydrogels for the development of controlled drug delivery system.

Acknowledgment

This work was supported by the Norwegian Research Council, contract number 191818/V30.

Notes and references

1) Biophysics and Medical Technology, Department of Physics, The Norwegian University of Science and Technology, NTNU, NO-7491 Trondheim, NORWAY. Fax: +47 73 59 77 ; Tel: +47 73 59 34 34; E-mail: bjorn.stokke@ntnu.no
[2]Department of Polymer Chemistry, Graduate School of Engineering, Kyoto University, Katsura, Nishikyo-ku, Kyoto, 615-8510, Japan
[3]ERATO Bio-nanotransporter Project, Japan Science and Technology Agency (JST), North Bldg., Kyodai Katsura Venture Plaza, 1-36, Goryo-Ohara, Nishikyo-ku, Kyoto, 615-8245, Japan.
 # These authors contributed equally to this work

1. V. P. Torchilin, *Journal of Controlled Release*, 2001, **73**, 137-172.
2. Y. Qiu and K. Park, *Advanced Drug Delivery Reviews*, 2001, **53**, 321-339.
3. B. G. De Geest, C. Déjugnat, E. Verhoeven, G. B. Sukhorukov, A. M. Jonas, J. Plain, J. Demeester and S. C. De Smedt, *Journal of Controlled Release*, 2006, **116**, 159-169.
4. S. V. Vinogradov, T. K. Bronich and A. V. Kabanov, *Advanced Drug Delivery Reviews*, 2002, **54**, 135-147.
5. A. V. Kabanov and S. V. Vinogradov, *Angew. Chem. Int. Ed.*, 2009, **48**, 5418-5429.
6. K. Raemdonck, J. Demeester and S. De Smedt, *Soft Matter*, 2009, **5**, 707-715.
7. C. Li and S. Liu, *Journal of Materials Chemistry*, 2010, **20**, 10716-10723.
8. Y. Zhao, C. Zheng, Q. Wang, J. Fang, G. Zhou, H. Zhao, Y. Yang, H. Xu, G. Feng and X. Yang, *Adv. Funct. Mater.*, 2011, **21**, 2035-2042.
9. T. Zhou, C. Xiao, J. Fan, S. Chen, J. Shen, W. Wu and S. Zhou, *Acta Biomaterialia*.
10. S.-i. Yusa, M. Sugahara, T. Endo and Y. Morishima, *Langmuir*, 2009, **25**, 5258-5265.
11. S. Argentiere, L. Blasi, G. Morello and G. Gigli, *The Journal of Physical Chemistry C*, 2011, **115**, 16347-16353.
12. T. Hirakura, Y. Nomura, Y. Aoyama and K. Akiyoshi, *Biomacromolecules*, 2004, **5**, 1804-1809.
13. J. He, X. Tong and Y. Zhao, *Macromolecules*, 2009, **42**, 4845-4852.
14. H. Kang, A. C. Trondoli, G. Zhu, Y. Chen, Y.-J. Chang, H. Liu, Y.-F. Huang, X. Zhang and W. Tan, *ACS Nano*, 2011, **5**, 5094-5099.
15. W. Wu, N. Mitra, E. C. Y. Yan and S. Zhou, *ACS Nano*, 2010, **4**, 4831-4839.
16. M. H. Xiong, Y. J. Li, Y. Bao, X. Z. Yang, B. Hu and J. Wang, *Adv Mater*, 2012, **24**, 6175-6180.
17. K. Akiyoshi, S. Deguchi, N. Moriguchi, S. Yamaguchi and J. Sunamoto, *Macromolecules*, 1993, **26**, 3062-3068.
18. T. Nishikawa, K. Akiyoshi and J. Sunamoto, *Journal of the American Chemical Society*, 1996, **118**, 6110-6115.
19. K. Akiyoshi, S. Kobayashi, S. Shichibe, D. Mix, M. Baudys, S. Wan Kim and J. Sunamoto, *Journal of Controlled Release*, 1998, **54**, 313-320.
20. R. Breslow and B. Zhang, *Journal of the American Chemical Society*, 1996, **118**, 8495-8496.
21. S. K. Rodal, G. Skretting, Ø. Garred, F. Vilhardt, B. van Deurs and K. Sandvig, *Molecular Biology of the Cell*, 1999, **10**, 961-974.
22. A. Tsamaloukas, H. Szadkowska, P. J. Slotte and H. Heerklotz, *Biophys. J.*, 2005, **89**, 1109-1119.
23. Y. Sasaki and K. Akiyoshi, *The Chemical Record*, 2010, **10**, 366-376.

24. U. Hasegawa, S.-i. Sawada, T. Shimizu, T. Kishida, E. Otsuji, O. Mazda and K. Akiyoshi, *Journal of Controlled Release*, 2009, **140**, 312-317.
25. T. Nochi, Y. Yuki, H. Takahashi, S.-i. Sawada, M. Mejima, T. Kohda, N. Harada, I. G. Kong, A. Sato, N. Kataoka, D. Tokuhara, S. Kurokawa, Y. Takahashi, H. Tsukada, S. Kozaki, K. Akiyoshi and H. Kiyono, *Nat Mater*, 2010, **9**, 572-578.
26. A. Shimoda, S.-i. Sawada and K. Akiyoshi, *Macromolecular Bioscience*, 2011, **11**, 882-888.
27. M. Fujioka-Kobayashi, M. S. Ota, A. Shimoda, K. Nakahama, K. Akiyoshi, Y. Miyamoto and S. Iseki, *Biomaterials*, 2012, **33**, 7613-7620.
28. K. Akiyoshi, A. Ueminami, S. Kurumada and Y. Nomura, *Macromolecules*, 2000, **33**, 6752-6756.
29. Y. Nomura, Y. Sasaki, M. Takagi, T. Narita, Y. Aoyama and K. Akiyoshi, *Biomacromolecules*, 2004, **6**, 447-452.
30. N. Inomoto, N. Osaka, T. Suzuki, U. Hasegawa, Y. Ozawa, H. Endo, K. Akiyoshi and M. Shibayama, *Polymer*, 2009, **50**, 541-546.
31. K. Gawel, D. Barriet, M. Sletmoen and B. T. Stokke, *Sensors*, 2010, **10**, 4381-4409.
32. S. Tierney, D. R. Hjelme and B. T. Stokke, *Analytical Chemistry*, 2008, **80**, 5086-5093.
33. S. Tierney, B. M. H. Falch, D. R. Hjelme and B. T. Stokke, *Anal. Chem.*, 2009, **81**, 3630-3636.
34. S. Tierney, S. Volden and B. T. Stokke, *Biosensors and Bioelectronics*, 2009, **24**, 2034-2039.
35. S. Tierney and B. T. Stokke, *Biomacromolecules*, 2009, **10**, 1619-1626.
36. M. Gao, K. Gawel and B. T. Stokke, *Soft Matter*, 2011, **7**, 1741-1746.
37. K. Gawel and B. T. Stokke, *Soft Matter*, 2011, **7**, 4615-4618.
38. S. Tierney, M. Sletmoen, G. Skjåk-Bræk and B. T. Stokke, *Carbohydr. Polym.*, 2010, **80**, 828-832.
39. K. Gawel, M. Gao and B. T. Stokke, *European Polymer Journal*, 2012, **48**, 1949-1959.
40. M. Gao, K. Gawel and B. T. Stokke, *Journal of Colloid and Interface Science*, 2013, **390**, 282-290.
41. N. Morimoto, T. Endo, Y. Iwasaki and K. Akiyoshi, *Biomacromolecules*, 2005, **6**, 1829-1834.
42. Y. Ohtani, T. Irie, K. Uekama, K. Fukunaga and J. Pitha, *Eur. J. Biochem.*, 1989, **186**, 17-22.
43. K. Akiyoshi, S. Deguchi, N. Moriguchi, S. Yamaguchi and J. Sunamoto, *Macromolecules*, 1993, **26**, 3062-3068.
44. G. M. Pavlov, E. V. Korneeva, N. A. Smolina and U. S. Schubert, *European Biophysics Journal with Biophysics Letters*, 2010, **39**, 371-379.
45. V. Prot, H. M. Sveinsson, K. Gawel, M. Gao, B. Skallerud and B. T. Stokke, *In preparation*, 2013.
46. J. Nishijo, S. Moriyama and S. Shiota, *Chem. Pharm. Bull. (Tokyo)*, 2003, **51**, 1253-1257.