

Polyrotaxane-Film Formation Observed by Surface Plasmon Spectroscopy

Keiko Tawa*, Norihito Kuboyama, Ahmed Saleh, and Mutsuo Tanaka

Research Institute for Cell Engineering (RICE), National Institute of Advanced Industrial Science and Technology (AIST),
1-8-31 Midorigaoka, Ikeda, Osaka 563-8577, Japan
FAX: 81-727-51-9628, e-mail:tawa-keiko@aist.go.jp

It has been known that poly (ethylene glycol) (PEG) can form a polyrotaxane with α -cyclodextrin (α -CD) due to the molecular recognition. In this study, a self-assembled-monolayer (SAM) composed of mixture thiols, MeAZOPEG6 and diluent thiol, was prepared, and the formation of polyrotaxane-ultrathin film with α -CD was investigated by the surface plasmon resonance spectroscopy (SPR). The large increase in the film thickness was observed after addition of α -CD solution. α -CD rings were found to form polyrotaxane-film selectively with MeAZOPEG6 regardless of the kind of the diluent thiol. The azobenzene moiety substituted at the end of MeAZOPEG6 is considered to be a trigger molecule to capture α -CD rings and then, CD rings can penetrate through a PEG chain. Furthermore, the formation of polyrotaxane-film could be partially controlled by UV light irradiation.

Key words: self-assembled-monolayer, SPR, polyrotaxane, photocontrol, PEG, cyclodextrin

1. INTRODUCTION

Rotaxane and polyrotaxane formation based on a host-guest intermolecular interaction has been studied as one of the most interesting and useful supramolecular architecture. Recently, it has been developed in various new fields such as nano-machine, molecular wire, shuttle,¹ and motor.² Polyrotaxane formation with polyethylene glycol (PEG) and α -cyclodextrins (α -CD) has been sufficiently studied by Harada et al.^{3,4} since '90s. They reported that the polyrotaxane is formed by the sequence of α -CD rings including two ethylene glycol units for one ring. In this study, we proposed a novel photocontrollable polyrotaxane-film using a self-assembled monolayer (SAM) of oligo (ethylene glycol) chain and α -CD. The novel compound⁵ is composed of (1) alkyl chain modified with a mercapt group to form a SAM, (2) oligo (ethylene glycol), and (3) an azobenzene moiety as a photoresponsible group. Here, a (pseudo-) polyrotaxane and an inclusion complex were distinguished. The polyrotaxane expresses the architecture as CD rings penetrate through the ethylene glycol chain, and the inclusion complex expresses the complex such as azobenzene capped with a CD ring. The polyrotaxane-film formation process was in situ observed by surface plasmon resonance spectroscopy (SPR) technique⁶ and its formation was controlled by UV light irradiation.

2. EXPERIMENTAL

2.1 Measurements

SPR

SPR set-up we use in this study is briefly explained. A He-Ne laser operating at 632.8 nm passes an optical chopper (used also as the reference for the lock-in amplifier) and, two polarizers for intensity and polarization control. By using a θ - 2θ goniometer (Sigma Koki), the light reflected at the substrate covered with a thin gold layer is monitored by a photo diode. When the reflectivity is observed at a fixed angle of incidence, any change of the interfacial architecture can be quantified by evaluating the resulting change of the reflected intensity. The employed sample cell is made of teflon and contains in- and outlets for the solutions. The

volume is about 100 μ L. It is sealed via a viton O-ring against the Au-coated high refractive glass slide (LaSFN9; Hellma Optik ($\epsilon' = 3.4$)) which is index-matched to the prism. Thin gold layer is prepared as the thickness is in 47 ± 3 nm by the gold sputtering. As for the preparation of SAM, ethanol solution of a new compound, MeAZOPEG6 (as shown below), was diluted by the ethanol solution of spacer thiol as the total thiols concentration was 1 mM. All measurements were performed at room temperature (22-23 °C). Saturated α -CD aqueous solutions (145mg/ml) were applied to the sample cell. The dissociation process was initiated by milli-Q water rinsing with a peristaltic pump.

The change of film thickness was estimated by using the software of *Winspall*⁷ with below values. The difference in the real part of dielectric constant, ϵ' , between solutions also induces the spectral shift in SPR curve. ϵ' of α -CD saturated aqueous solution is higher (1.815) than that of milli-Q water (1.770) as evaluated from a critical angle. As the most appropriate values of ϵ' for SAM and α -CD, 2.2 and 2.6 were used, respectively.

FTIR-RAS

The composition of SAM mixing MeAZOPEG6 (as shown below) with diluent thiol was investigated by FTIR-reflection absorption spectroscopy (RAS) using the FT-IR instrument (FTS-175C, Bio-Rad) equipped with a specular reflectance accessory of incident angle fixed at 80 degree and a MCT detector. The integration and resolution were 20,000 times and 4 cm^{-1} , respectively.

2.2 Synthesis

All chemicals for synthesis were of available purity and used without further purification. The synthesis was carried out according to the outline as shown in insets.

MeAZOPEG6

10-Bromo-1-decanol (20 mmol), 4-4'-biphenol (40 mmol), and acetonitrile (1L) were put to a three-necked flask under nitrogen atmosphere, and then, K_2CO_3 (200 mmol) and NaI (2 mmol) were added to the mixture. The reaction mixture was refluxed under nitrogen atmosphere for 24 hrs. The obtained residue by solvent evaporation was rinsed with aq.

NaOH (5 wt%) to remove excess 4,4'-biphenol, and then, neutralized with aq. HCl (5 wt%). To the obtained solid, THF was added, and insoluble materials were filtrated off. Crude product **a-1** was obtained by solvent evaporation in 74% yield, and was used for the subsequent reaction without purification.

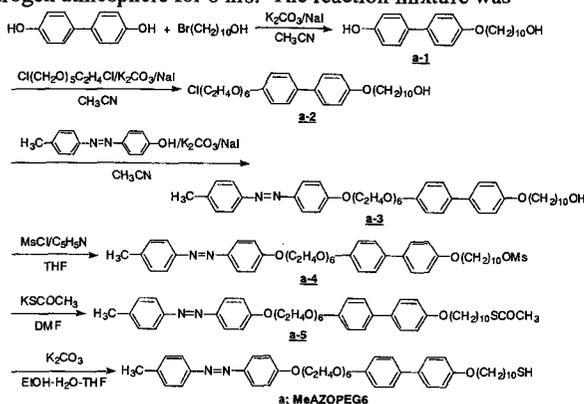
Under nitrogen atmosphere, **a-1** (1 mmol), hexaethylene glycol dichloride (3 mmol), and acetonitrile (50 mL) were put to a three-necked flask, and K_2CO_3 (20 mmol) and NaI (0.6 mmol) were added to the mixture. The reaction mixture was refluxed under nitrogen atmosphere for 48 hrs. The reaction mixture was poured into water, and the product was extracted with $CHCl_3$. The product **a-2** was purified by gel permeation chromatography (GPC) (46% yield).

Prepared tolylazophenol (1 mmol), **a-2** (0.5 mmol), and acetonitrile (50 mL) were placed to a three-necked flask under nitrogen atmosphere, and then, K_2CO_3 (5 mmol) and NaI (0.2 mmol) were added to the mixture. The reaction mixture was refluxed under nitrogen atmosphere for 24 hrs. The reaction mixture was poured into water, and the product was extracted with $CHCl_3$. The product **a-3** was purified by GPC (68% yield).

To a three-necked flask, **a-3** (0.5 mmol), pyridine (10 mmol), and THF (50 mL) were placed. Methane sulfonylchloride (10 mmol) was added to the mixture, and the reaction mixture was stirred for 48 hrs at room temperature. The reaction mixture was poured into water, and the product was extracted with ethyl acetate. The organic layer was rinsed with aq. HCl. After solvent evaporation, the product **a-4** was purified by GPC (85% yield).

Under nitrogen atmosphere, **a-4** (0.5 mmol) and dry DMF (50 mL) were put to a three-necked flask, and then, potassium thioacetate (5 mmol) was added to the mixture. The reaction mixture was stirred for 6 hrs at room temperature. The reaction mixture was poured into water, and the product was extracted with $CHCl_3$, twice. The product **a-5** (quantitative yield) obtained after drying under vacuum condition was used for the subsequent reaction without purification.

To a mixture of ethanol (40 mL), water (20 mL), and THF (60 mL) in a three-necked flask, **a-5** (0.5 mmol) was added under nitrogen atmosphere, and then, K_2CO_3 (3.62 mmol) was added. The reaction mixture was refluxed under nitrogen atmosphere for 6 hrs. The reaction mixture was



Scheme 1 Synthesis of MeAZOPEG6

poured into aq. HCl, and the product was extracted with $CHCl_3$. The product MeAZOPEG6⁸ obtained by solvent evaporation was purified by GPC (orange viscous oil, 56% yield).

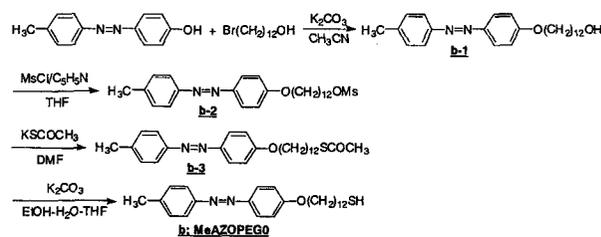
MeAZOPEGO

12-Bromo-1-dodecanol (2 mmol), tolylazophenol (2 mmol), and acetonitrile (150 mL) were put to a three-necked flask under nitrogen atmosphere, and then, K_2CO_3 (200 mmol) was added to the mixture. The reaction mixture was refluxed under nitrogen atmosphere for 24 hrs. The residue obtained by solvent evaporation was treated with $CHCl_3$ and aq. HCl to extract the product. The product **b-1** (quantitative yield) obtained after solvent evaporation and dryness under vacuum condition was used for the subsequent reaction without purification.

At room temperature, **b-1** (2 mmol), pyridine (40 mmol), and THF (150 mL) were placed to a three-necked flask, and then, THF solution (40 mL) of methane sulfonylchloride (40 mmol) was added dropwise to the mixture. The reaction mixture was stirred for 48 hrs at room temperature. The reaction mixture was poured into aq. HCl, and the product was extracted by benzene. The organic layer was rinsed with aq. HCl. The product **b-2** obtained by solvent evaporation was purified by GPC (51% yield).

Under nitrogen atmosphere, **b-2** (1.4 mmol) and dry DMF (50 mL) were put into a three-necked flask at room temperature, and then, potassium thioacetate (14 mmol) was added. The reaction mixture was stirred for 6 hrs. The reaction mixture was poured into aq. HCl, and the product was extracted with $CHCl_3$. The product **b-3** (79% yield) obtained by solvent evaporation was dried under vacuum condition, and used for the subsequent reaction without purification.

To a mixture of ethanol (40 mL), water (20 mL), and THF (60 mL) in a three-necked flask, **b-3** (1 mmol) was added under nitrogen atmosphere, and then, K_2CO_3 (7.2 mmol) was added. The reaction mixture was refluxed for 6 hrs. After cooling, the reaction mixture was poured into aq. HCl, and the product was extracted with $CHCl_3$. Purification of the product by GPC afforded MeAZOPEG0⁹ in 46% yield as orange solid.



Scheme 2 Synthesis of MeAZOPEG0

3. RESULTS AND DISCUSSION

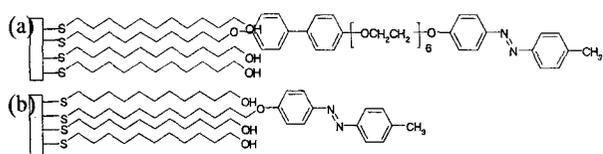
3.1 Composition of a Self-Assembled-Monolayer

The molecular density of MeAZOPEG6 in the SAM mixed with diluent spacer thiol, i.e., dodecane thiol (HSC12) or 11- mercapto-1-undecanol (HSC11OH) was investigated in order to acquire the information about whether MeAZOPEG6 chain has the enough space to catch CD in rotaxane-film formation. The composition of the mixed SAM was estimated by FTIR-RAS. Mixture ratio of the thiol solutions was not equal to the composition ratio in the SAM

Table 1. The time-dependent film thickness after addition of α -CD and γ -CD solutions, dissociation time of CD from the surface by milli-Q rinse, and the thickness of residue after rinse.

	(in α -CD) Thickness / nm			Dissociation time / min	Residue / nm	(in γ -CD) Thickness / nm			Dissociation time/min	Residue / nm
	10min	2 hrs	20hrs			10min	2 hrs	20hrs		
HSC12	1.9±0.2	2.6±0.2	3.6±0.1	2.0±0.5	0.7±0.2	9.0±0.2	9.7±0.2	10.9±0.2	1.0±0.5	8.9±0.2
HSC11OH	0.1±0.1	0.5±0.1	0.5±0.2	2.5±0.5	0.1±0.1	0	0	0	-	0
PEG120	9.8±0.2	10.0±0.2	-	5.0±1.0	0	0	0	0	-	0
1:9-MeAZOPEG6										
:HSC12	2.0±0.1	2.0±0.1	2.0±0.2	5.0±1.0	0	0	0	0	-	0
1:9-MeAZOPEG6										
:HSC11OH	1.9±0.1	2.0±0.1	2.2±0.1	4.5±0.5	0	0	0	0	-	0

The layer thickness was evaluated by fitting the SPR spectra to Fresnel's theoretical curve using *Winspall*.⁷

**Fig.1** Mixed SAM of (a)MeAZOPEG6 and (b)MeAZOPEG0.

because of the different reaction kinetics of two kinds of thiols. The mixed solution of 1:9-MeAZOPEG6:HSC11OH was found to build SAM containing 75%±5% of spacer thiol. The FTIR-RAS measurement also supplied that the density of a total alkyl chain (of spacer thiol and MeAZOPEG6) on the gold surface decreased as the composition ratio of MeAZOPEG6 increased. It is considered to be due to the expansion of PEG chain. As a result of IR bands assigned to CH₂ stretching and phenyl ring, the chain density of 1:9-SAM is 63% of that of HSC11OH-SAM. The SAM composed of only a long alkyl chain has been known to take a hexagonal lattice¹⁰ (nearest neighbor spacing: 0.5 nm) on Au (111) surface. The spacing value is assume to be equal to that in HSC11OH-SAM. Therefore, the nearest neighbor spacing of MeAZOPEG6 chain in 1:9-SAM is estimated as ca. 1.2 nm under the assumption of molecular dispersion. It is most suitable to capture α -CD rings densely among the various mixed SAMs, considering the molecular size of α -CD with outer diameter¹¹ of 1.3 nm.

3.2 Interaction of α -cyclodextrin with the interface

The interaction of α -CD with a solid surface has widely been studied and α -CD has been known to favor the adsorption by lying-form on the apolar surface because of the hydrophobic wall.¹² The interaction of α -CD with the SAM interface of hydrophobic HSC12 and hydrophilic HSC11OH is summarized in Table 1. CD molecules are considered to adsorb on the hydrophobic HSC12 surface and aggregate each other.¹³ On the HSC11OH surface, the increase in film thickness is below the size of one layer of α -CD molecules. On the monolayer with a longer ethylene glycol chain prepared from mercapto-PEG120 (NOF corporation, SUNBRIGHT ME-050SH Mw=5000), the film thickness immediately increased more than 10nm after addition of saturated α -CD aqueous solution, due to the extension of a PEG chain accompanying the formation of polyrotaxane. Further, the dissociation time by rinse is longer than that for the HSC12 and HSC11OH surface.

Harada et al. reported⁴ that the yield of polyrotaxane between PEG and α -CD depends on the molecular weight in the aqueous solution, i.e., oligo (ethylene glycol) with less than 3 units cannot form the polyrotaxane and the yield increases with the number of ethylene glycol unit. The monolayer of PEG120 is considered to be sufficient unit number to form the polyrotaxane-film, though the condition of polyrotaxane-film formation should be different from that in their work under homogeneous solution state.

3.3 Formation of polyrotaxane-film

In advance of the experiment of polyrotaxane-film formation using MeAZOPEG6, the polyrotaxane formation between MeAZOPEG6 (not film) and α -CD was measured in the α -CD aqueous solution through turbidity. MeAZOPEG6 is so difficult to dissolve in water, but the solution was immediately turbid and then participation was also observed. It is considered that the polyrotaxane was formed in the α -CD solution and aggregated each other.

SPR spectra depicted in Figure 2 indicate polyrotaxane-film formation between 1:9-MeAZOPEG6:HSC11OH film and α -CD. The spectral shift from (a) (in water before injection of α -CD) to (b) (in CD solution 2hrs after injection) does not directly correspond to the increase in film thickness, because of the difference in the refractive index between milli-Q water and α -CD solution. Therefore, the increase in film thickness due to the formation of polyrotaxane-film can be evaluated by the shift from (c) (the simulation curve before injection of α -CD in which the refractive index of water was changed to that of α -CD solution) to (b). As found from Table 1, the increase was ca. 2.0 nm in the 1:9-MeAZOPEG6:HSC12 surface and was almost equal to that in the 1:9-SAM using HSC11OH. Accordingly, CD molecules could selectively interact with MeAZOPEG6 chain in the 1:9-SAM regardless of the kind of a hydrophilic or hydrophobic spacer thiol. The increase in film thickness and longer dissociation time could be due to the extension of hexaethylene glycol chain by the polyrotaxane-film formation. Furthermore, the 1:9-MeAZOPEG0:HSC11OH SAM was also prepared (Fig. 1(b)). The film thickness which increased after adding α -CD was 0.9±0.1 nm even after 24hrs. The small increase may be mainly due to the formation of inclusion complex on the MeAZOPEG0, i.e., azobenzene moiety is capped with α -CD ring.¹⁴ Therefore, 4-methyl azobenzene of MeAZOPEG6 may be a trigger molecule to capture α -CD rings which can

pass through a hexaethylene glycol chain.

The interaction of 1:9-SAM with γ -CD was also examined by the SPR. As described in Table 1, MeZOPEG6 can't recognize γ -CD. The polyrotaxane-film formation with γ -CD was not observed in the PEG120 monolayer, either.

3.4 Photocontrol of polyrotaxane formation

A photoswitchable rotaxane based on stilbene photoisomerization was already reported¹⁵ in a solution by Tokunaga et al. Here, we aim to invent the polyrotaxane-ultrathin film controllable by UV light. When UV light ($\lambda = 300$ -400 nm) irradiated to the 1:9-MeAZOPEG6:HSC11OH SAM, the absorption peak assigned to π - π^* of *trans* form was found to decrease as a result of partial isomerization. During UV irradiation, the increase in the film thickness was only 1.6 ± 0.1 nm at 10min after adding α -CD solution and it was still fixed 2hrs afterward. Such a smaller increase is considered to be due to that the *cis* isomers block α -CD rings penetrating through an ethylene glycol chain. Furthermore, dissociation time in the 1:9 SAM under UV is ca. 1 min shorter than that without UV. The formation of polyrotaxane-film was able to be controlled partially by UV light irradiation.

4. CONCLUSION

Using a novel compound, MeAZOPEG6, synthesized in this study, self-assembled-monolayer was prepared on the thin gold layer. MeAZOPEG6 in the mixed SAM selectively formed polyrotaxane-film with α -CD regardless of the kind of diluent thiol. The azobenzene derivatives modified at the chain-end was found to make a key role of photocontrolling polyrotaxane-film formation. Under the UV irradiation, azobenzene derivatives isomerized to *cis* form partially prevented forming the polyrotaxane-film. We will develop the polyrotaxane-SAM in which remarkable photocontrol is possible.

Acknowledgment.

K.T. thanks Grants-in-Aid for Scientific Research (Japan Society for the Promotion of Science) for financial support, and Prof. Dr. Wolfgang Knoll and Mr. Andreas Scheller of Max-Planck-Institute for Polymer Research for constructing SPR set-up. We also thank Dr. Toshiyuki Mihara and Ms. Sachiko Namba for experimental cooperation.

References

- (a) P. L. Anelli, N. Spencer, and J. F. Stoddart, *J. Am. Chem. Soc.* **113**, 5131-5133 (1991). (b) H. Murakami, A. Kawabuchi K. Kotoo, M. Munitake, and N. Nakashima, *J. Am. Chem. Soc.* **119**, 7605-7606 (1997).
- (a) V. Balzani, A. Credi, F. M. Raymo, and J. F. Stoddart, *Angew. Chem., Int. Ed.* **39**, 3348-3391(2000). (b) H. Yamaguchi, M. Kamachi, and A. Harada, *Angew. Chem., Int. Ed.* **39**, 3829-3831 (2000). (c) A. Harada, *Adv. Polym. Sci.* **133**, 141-191 (1997). (d) S.A.Nepogodiev and J. F. Stoddart, *Chem. Rev.* **98**, 1959-1976 (1998). (e) Y. Kawaguchi and A. Harada, *J. Am. Chem. Soc.*, **122**, 3797-3798 (2000).
- A. Harada, Synthesis of Polyrotaxanes. In Synthesis of Polymers; Schlüter, A.-D., Ed.; Wiley-VCH: Weinheim, 1999.
- (a) A. Harada, J. Li, and M. Kamachi, *Nature*, **364**, 516-518 (1993). (b) A. Harada, J. Li, and M. Kamachi, *Nature*, **370**, 126-128 (1994). (c) A. Harada, J. Li, and M. Kamachi, *Macromolecules*, **27**, 4538-4543 (1994). (d) M. Okada and A. Harada, *Macromolecules*, **36**, 9701-9703 (2003).
- K. Tawa and M. Tanaka, Japan Patent, Tokugan2003-287629 'Azoaryl mercaptoalkyl polyethylene glycol' (2003).
- W. Knoll, *Annu. Rev. Phys. Chem.* **49**, 569-638 (1998).
- WINSPALL is a software which computes the reflectivity of multilayer systems. It is based on the Fresnel equations and the matrix formalism. It is available for use from 'http://www.mpip-mainz.mpg.de/documents/akkn/index.html'.
- ¹H-NMR; (CDCl₃, 400 MHz) $\delta = 1.3$ -2.0 (16H, m, CH₂), 2.39 (3H, s, CH₃), 2.52 (2H, q, J=6.9 Hz, CH₂S), 3.6-3.9 (20H, m, CH₂O), 3.97 (2H, t, J=6.4 Hz, PhOCH₂), 4.15 (2H, t, J=4.8 Hz, PhOCH₂), 4.19 (2H, t, J=4.6 Hz, PhOCH₂), 6.93 (2H, d, J=8.8 Hz, ArH), 6.95 (2H, d, J=8.0 Hz, ArH), 7.01 (2H, d, J=8.8 Hz, ArH), 7.29 (2H, d, J=8.0 Hz, ArH), 7.4-7.5 (4H, m, ArH), 7.78 (2H, d, J=8.0 Hz, ArH), 7.88 (2H, d, J=8.8 Hz, ArH).
- ¹H-NMR; (CDCl₃, 400 MHz) $\delta = 1.2$ -1.9 (20H, m, CH₂), 2.42 (3H, s, CH₃), 2.52 (2H, q, J=5.9 Hz, CH₂S), 4.03 (2H, t, J=6.6 Hz, PhOCH₂), 6.99 (2H, d, J=8.8 Hz, ArH), 7.29 (2H, d, J=8.0 Hz, ArH), 7.78 (2H, d, J=8.4 Hz, ArH), 7.89 (2H, d, J=8.8 Hz, ArH).
- H. Takiguchi, K. Sato, T. Ishida, K. Abe, K. Yase, and K. Tamada, *Langmuir*, **16**, 1703-1710 (2000).
- Y. Sueishi and H. Hishikawa, *Int. J. Chem. Kinet.*, **34**, 481-487 (2002).
- P. Bortolus and S. Monti, *J. Phys. Chem.* **91**, 5046-5050 (1987).
- Y. Tokunaga, K. Akasaka, K. Hisada, Y. Shimomura and S. Kakuchi, *Chem. Comm.* 2250-2251 (2003).
- H. Shigekawa, T. Morozumi, and M. Komiyama, *J. Vac. Sci. Technol. B.*, **9**, 1189-1192 (1991).
- L. Pospisil and M. Svestka, *J. Electanal. Chem.*, **426**, 47-53 (1997).

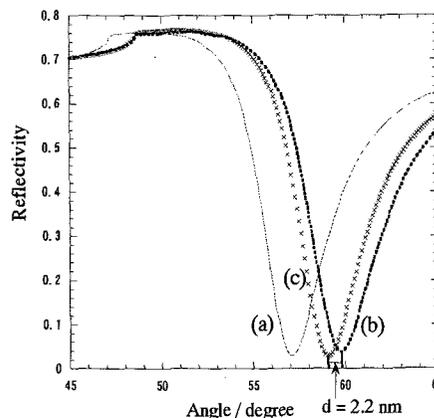


Fig. 2. SPR curves measured for 1:9-SAM: (a) in water before injection of α -CD solution (—), (b) in α -CD solution 2hr after injection (···), and (c) simulation curve (changed to a refractive index of the saturated α -CD solution) before injection (x x x).