Investigation of the Complexation Behavior of a New Polymer with Cyclodextrin and Crown Ether Side Groups by Fluorescence Spectroscopy

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Summary: A thermoresponsive terpolymer of *N*-isopropylacrylamide (NIPA), 6-*N*-allylamino-6-deoxy-β-cyclodextrin (AA-β-CD) and 4-acryloylamido-benzo-15-crown-5 (AAB-15-crown-5) was synthesized by free radical polymerization to a combined supramolecular host system. The complexation of a fluorescent dye, namely ammonium 8-anilino-1-naphthalenesulfonate (ANS), as guest molecule was investigated via fluorescence spectroscopy. In presence of the terpolymer, the fluorescence of ANS was highly increased compared to native β-CD and the copolymers of NIPA and AA-β-CD or AAB-15-crown-5.

Keywords: crown ether; cyclodextrin; host-guest systems; PNIPA; radical polymerization

Introduction

Cyclodextrins (CDs) and crown ethers (CEs) are common and well studied examples for supramolecular host molecules. CDs are cyclic oligo-saccharides building a truncated cone with a hydrophobic cavity and a hydrophilic outside. Due to their ability to incorporate various nonpolar organic molecules, they are useful in applications such as enantioselective separation[1,2] or for the elimination of bitter tastes of foods.[3] In our studies the widely investigated β-CD, which consists of 7 glucopyranose units was used. CEs are cyclic oligo-ethers, which have a polar or nonpolar interior in dependence of the polarity of the surrounding solvent. Thus, they are able to build complexes with cations as well as neutral molecules. They find application in phase transfer catalysis and anion activation.[1] Also a combination of both host molecules as a kind of complexation unit is already known in literature.[4] This CE-tethered CD builds a complex with 8-anilino-1-naphthalenesulfonate (ANS) and its counter ion. ANS is a fluorescent dye (Scheme 1) and its fluorescence is enhanced when it is located in a less polar environment than water, such as in the cavity of a CD molecule.

The CE-cation complex is able to further enhance the shielding of ANS from water, due to cooperative attracting interactions.[4] This causes a higher fluorescence intensity compared to ANS included in a CD cavity without CE.

Host molecules like CD and CE in combination with polymers are of broad interest for various applications. In literature different kinds of polymers bearing CD or CE as side groups to combine the complexation properties of the host molecule and the properties of the polymer are reported.[5,6] Especially stimuli-responsive polymers are applied in fields such as pharmaceutical,[7] food and textile industry[8] or as stationary phase in chromatography.[2,9] In case of stationary phases, CD polymers play an important role due to their ability to separate enantiomers. A well known stimuli-responsive polymer is *N*-isopropylacrylamide (NIPA), which reacts on temperature changes. In water, it exhibits a lower critical solution temperature

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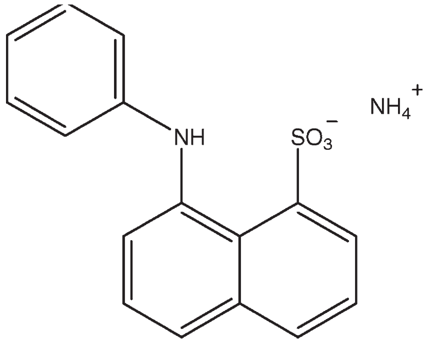


Scheme 1.
Chemical structure of ammonium 8-anilino-1-naphthalenesulfonate (ANS).

(LCST) of around 33 °C.[10] Hence, underneath the LCST the polymer is completely dissolved while above the LCST, it becomes insoluble in water. This behavior is useful for instance in drug delivery systems.

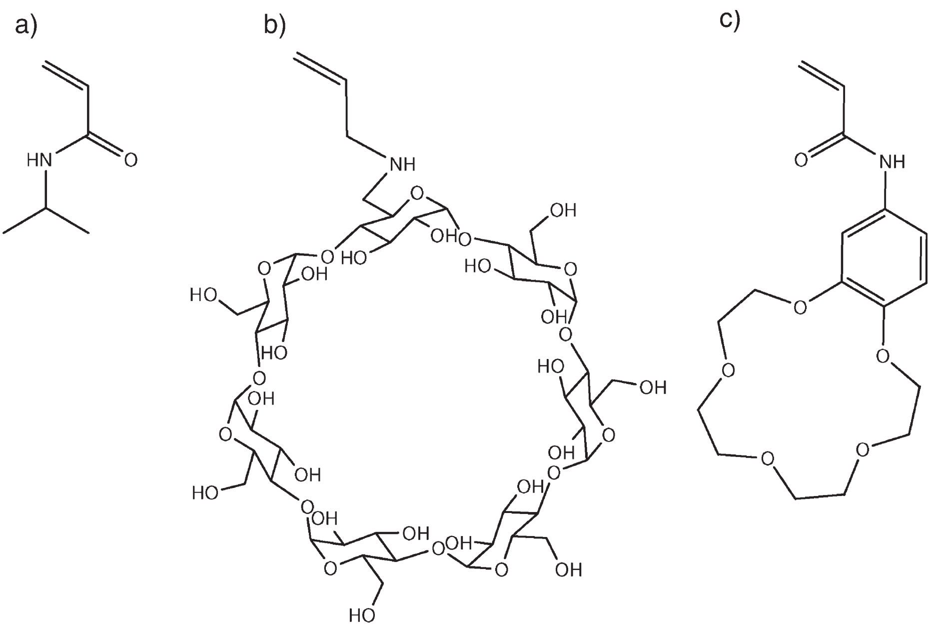
We synthesized a thermoresponsive polymer containing the above mentioned combination of β-CD and CE derivatives, namely 6-*N*-allylamino-6-deoxy-β-cyclodextrin (AA-β-CD) and 4-acryloylamidobenzo-15-crown-5 (AAB-15-crown-5), and investigated the complexation of ANS via fluorescence spectroscopy in an aqueous sodium phosphate buffer. Beside a constant pH, the buffer has the advantage of delivering sodium, which is an ideal guest molecule for CE and can act as counter ion for ANS. The monomers we used, are shown in Scheme 2.

A thermoresponsive polymer was used, because it will enable us to control the complexation of guest molecules (Scheme 3), which will be investigated in the future.

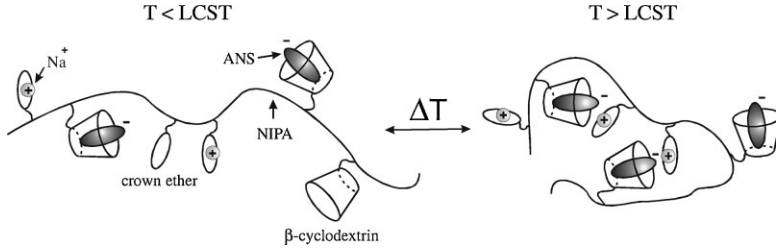
Experimental Part

Materials

β-cyclodextrin hydrate (99.0 w%), sodium dihydrogen phosphate (99%), ANS (97%) and *p*-toluenesulfonyl chloride (99%) were purchased from Fluka. Acetonitrile (p.a.) and allylamine were purchased from Merck. Sodium phosphate dibasic (99%), ammonium persulfate (APS) (98+%), N,N,N′,N′-tetramethylethylenediamine (TEMED)



Scheme 2.
Chemical structure of the used monomers: a) *N*-isopropylacrylamide (NIPA), b) 6-*N*-allylamino-6-deoxy-β-cyclodextrin (AA-β-CD) and c) 4-acryloylamidobenzo-15-crown-5 (AAB-15-crown-5).



Scheme 3.
Schematic representation of the complexation process of ANS by a polymer bearing cyclodextrin and crown ether as side groups in dependence of the temperature.

(99%) and methanol (99.9% A.C.S.) were purchased from Sigma-Aldrich. NIPA (99%, stabilized) and AAB-15-crown-5 (99%) were purchased from Acros.

NIPA was recrystallized before use, all other chemicals were used as received without further purification.

Synthesis of Monomer and Polymers

Allylamino-β-CD [AA-β-CD]: The *β*-CD monomer AA-*β*-CD was synthesized in a two step synthesis from *β*-CD. Mono-6-deoxy-6-(*p*-toluenesulfonyl)-*β*-CD was synthesized according to ref.[11] and was obtained in 8% yield. In a second step AA-*β*-CD was synthesized according to ref.[12] and was obtained in 74% yield. The products were characterized by ^{1}H- and ^{13}C-NMR spectroscopy.

Poly(NIPA) [PNIPA]: 2.21 mmol (0.25 g) NIPA were dissolved in 3.96 mL deionizied water and 1.01 mL methanol. The reaction mixture was purged with nitrogen for 10 minutes and 0.015 mmol (0.0035 g) APS in 1.18 mL deionizied water and 0.039 mmol (0.0045 g) TEMED in 1.05 mL deionizied water were added. The reaction vessel was sealed and kept at room temperature for 15 h. The reaction solution was dialyzed for 3 days in water/methanol (v/v = 235mL/15mL) and additional 4 days in pure water. Afterwards the product was isolated via freeze-drying.

Yield: 76%, ^{1}H-NMR (400 MHz, D_2O):*δ* (ppm) = 0.70-2.17 (m, 9 H, C$\underline{H}_3$, C$\underline{H}$-C$\underline{H}_2$), 3.65-3.96 (m, 1 H, C$\underline{H}(CH_3)_2$).

Poly(NIPA-co-AA-β-CD) [P(NIPA-co-CD)]: 0.12 mmol (0.1441 g) AA-*β*-CD were dissolved in a mixture of 3.96 mL deionizied water and 1.01 mL methanol. 2.21 mmol (0.25 g) NIPA were added and the solution purged with nitrogen for 10 minutes. Initiator and accelerator amounts, the reaction time as well as purification and isolation were performed as mentioned above. The incorporation of *β*-CD into the polymer was determined by ^{1}H-NMR spectroscopy.

Yield: 59%, ^{1}H-NMR (400 MHz, D_2O): *δ* (ppm) = 0.75-2.22 (m, C$\underline{H}_3$ (NIPA), C$\underline{H}$-C$\underline{H}_2$ (NIPA + AA-*β*-CD: backbone)), C(7)$\underline{H}$ (AA-*β*-CD)), 3.00-4.02 (m, C$\underline{H}(CH_3)$ (NIPA), C(2)$\underline{H}$, C(3)$\underline{H}$, C(4)$\underline{H}$, C(5)$\underline{H}$, C(6)$\underline{H}_2$ (AA-*β*-CD)), 4.94-5.03 (m, 7 H, C(1)$\underline{H}$ (AA-*β*-CD)).

Poly(NIPA-co-AAB-15-crown-5) [P(NIPA-co-CE)]: 0.12 mmol (0.0414 g) AAB-15-crown-5 were dissolved in 1.01 mL methanol and mixed with a solution of 2.21 mmol NIPA in 3.96 mL deionizied water. The reaction solution was purged with nitrogen for 10 minutes. Initiator and accelerator amounts, the reaction time as well as purification and isolation were performed as mentioned above. The incorporation of AAB-15-crown-5 into the polymer was determined by ^{1}H-NMR spectroscopy.

Yield: 71%, ^{1}H-NMR (400 MHz, D_2O): *δ* (ppm) = 0.34-2.43 (m, 217 H, C$\underline{H}_3$ (NIPA), C$\underline{H}$-C$\underline{H}_2$ (NIPA + AAB-15-crown-5)), 3.34-4.19 (m, 38 H, C$\underline{H}(CH_3)$ (NIPA), C$\underline{H}_2$ (AAB-15-crown-5)), 6.59-7.23 (m, 3 H, C$\underline{H}$(arom., AAB-15-crown-5)).

Poly(NIPA-co-AA-β-CD-co-AAB-15-crown-5) [terpolymer]: 0.12 mmol (0.0414 g)

AAB-15-crown-5 were dissolved in 1.01 mL methanol and added to a solution of 0.12 mmol (0.1441 g) AA-β-CD and 2.21 mmol (0.25 g) NIPA in 3.96 mL deionizied water. The reaction solution was purged with nitrogen for 10 minutes. Initiator and accelerator amounts, the reaction time as well as purification and isolation were performed as mentioned above. The incorporation of AAB-15-crown-5 and AA-β-CD into the polymer was determined by ^{1}H-NMR spectroscopy.

Yield: 51%, ^{1}H-NMR (400 MHz, D_2O): δ (ppm) = 0.50-2.45 (m, C$\underline{H}_3$ (NIPA), C$\underline{H}$-C$\underline{H}_2$ (NIPA + AAB-15-crown-5 + AA-β-CD: polymer backbone), C(7)$\underline{H}$ (AA-β-CD)), 3.00-4.25 (m, C$\underline{H}$(CH_3) (NIPA), O-C$\underline{H}_2$-C$\underline{H}_2$ (AAB-15-crown-5), C(2)$\underline{H}$, C(3)$\underline{H}$, C(4)$\underline{H}$, C(5)$\underline{H}$, C(6)$\underline{H}_2$ (AA-β-CD)), 4.88-5.05 (m, 7 H, C(1)$\underline{H}$ (AA-β-CD)), 6.67-7.25 (m, 3 H, C$\underline{H}$(arom., AAB-15-crown-5)).

^{1}H-NMR spectra were recorded with a BRUKER Digital FT-NMR 'AVANCE 400'.

Fluorescence spectra were recorded with a SPEX FluoroLog II spectrometer at 18 °C to assure stable solutions of the PNIPA-containing polymers. The excitation wavelength was 350 nm. The fluorescence behavior of ANS in the presence of the polymers was determined by varying the polymer concentration while keeping the ANS concentration (250 μmol/L) fixed. An aqueous NaH_2PO_4/Na_2HPO_4 buffer solution (pH = 7.2) was used as a solvent.

Results and Discussion

Polymer Characterization

To determine the molar mass SEC with THF as solvent was used. All polymeric products exhibit a sufficiently high molecular weight. Due to the lack of a polymer standard for calibration, the molar masses could not be determined exactly. Using polystyrene (PS) as a standard, the molar mass of PNIPA was calculated to M_w = 886,000 g/mol. Unfortunately the copolymers and the terpolymer show attractive interactions with the stationary phase which leads to rather low molar masses. Table 1 shows the molar masses resulting from the maxima of the elution diagrams. Again PS was used as a standard for calibration.

Please note, that these are estimated values and the absolute values should be higher.

The conversion of the monomers to polymer has not been determined yet, because the small amount of obtained product did not allow a purification of the polymers by precipitation. Instead dialysis has been used which is known to result in a significant material loss. However, the yields between 51 and 76% of the obtained polymers after dialysis indicate that the conversion for all reactions was rather high.

Incorporation of AA-β-CD and AAB-15-crown-5 was determined by ^{1}H-NMR spectroscopy. To calculate the incorporation characteristic NMR-bands for every monomer were chosen, as shown in Figure 1.

The results are listed in Table 2.

In the polymerization reaction 5 mol% AA-β-CD (relative to NIPA) were added. However, the table shows a very low yield of incorporated AA-β-CD of only 0.23 mol% of the monomer. This is due to the low copolymerization tendency of allylamines in general. AAB-15-crown-5, which was also added as 5 mol%, is incorporated to 4.62 mol% due to its highly reactive acrylamide group.

Table 1.
estimated molar masses determined by SEC.

	P(NIPA-*co*-CE)	P(NIPA-*co*-CD)	terpolymer
molar mass [g/mol]	220.000	125.000	14.000

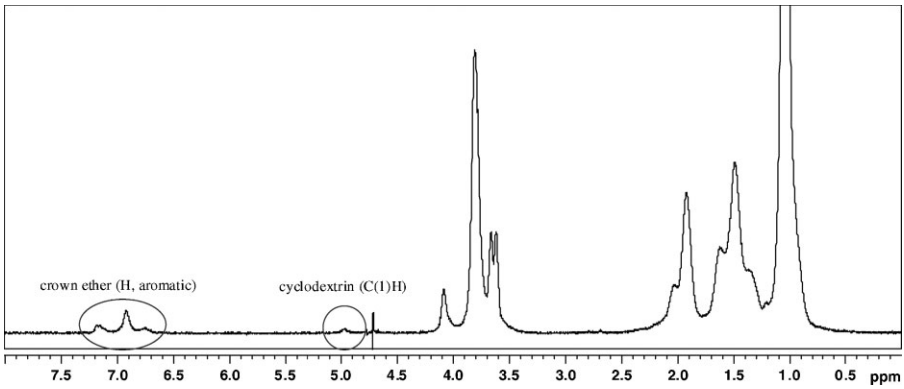


Figure 1.
^{1}H-NMR spectrum of the terpolymer. The characteristic bands for the monomers are marked.

Table 2.
Copolymer and terpolymer composition determined by ^{1}H-NMR spectroscopy.

Monomer incorporation relative to NIPA [mol-%]		
	AAB-15-crown-5	AA-β-CD
P(NIPA-*co*-CE)	4.3	—
P(NIPA-*co*-CD)	—	0.23
Terpolymer	4.62	0.15

Fluorescence Spectroscopy

As mentioned above, CE-tethered CDs are able to enhance the fluorescence of ANS more than native β-CDs do. This effect is explained by a cooperative attracting interaction between CE and CD.[4]

In case of CD and CE linked to a polymer backbone, the distances between them are much larger than in a tethered molecule. Nevertheless, the fluorescence spectra of ANS (Figure 2) show a significant intensity increase in presence of the terpolymer compared to native β-CD, which indicates the interacting of CD and CE as complexation unit in a polymer as well.

The complexation ability of the CD units towards ANS in the synthesized terpolymer and P(NIPA-*co*-CD) as well as that of native β-CD is depicted in Figure 3a). The normalized maximum fluorescence intensities increase linearly with increasing β-CD concentration. From the slopes in Figure 3a) the complexation ability of the terpolymer is 28 times higher than that of native β-CD and 2.5 times higher than that of P(NIPA-*co*-CD). This strong effect indicates a synergistic effect of CD and CE units in the terpolymer. Although the distances of CE and CD in a polymer may not be as short as in a tethered molecule, the incorporation in a polymer coil is sufficient to get cooperative interactions. We do not calculate any equilibrium constants in this paper, because the complexation mechanism is not known in detail yet.

The fact, that the copolymer P(NIPA-*co*-CD) also increases the complexation 11-fold relative to native β-CD shows the positive effect of the PNIPA backbone on the complexation. This is probably due to a shielding effect of the polymer coil. To assure that the observed complexation increase is not solely based on this polymeric shielding, we also investigated the complexation of ANS in pure PNIPA and P(NIPA-*co*-CE).

As shown in Figure 3b), again the terpolymer has the strongest complexation ability whereas PNIPA has only a marginal effect on the fluorescence intensity of ANS. Please note that even though one might get the impression that the effect of PNIPA is comparable to the one of native β-CD (Figure 3a), this is not in the least the case due to the very different concentration scales of both diagrams. Native β-CD in similar weight concentration to the polymer concentrations would exhibit a much

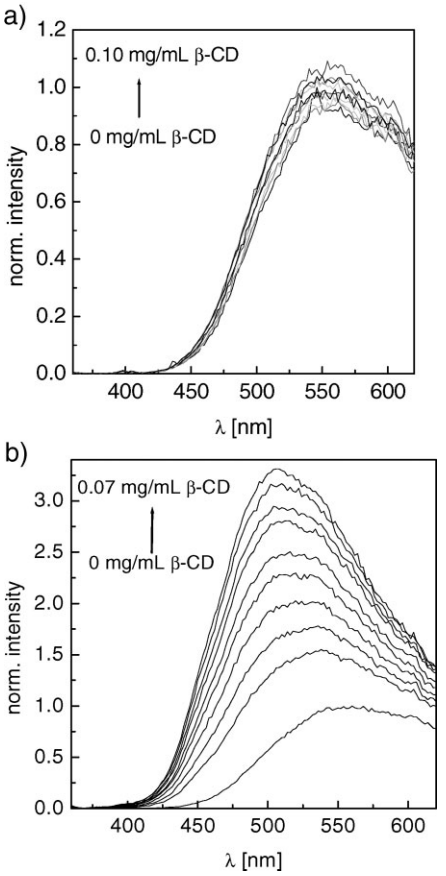


Figure 2.
Normalized fluorescence emission spectra of ANS in presence of increasing concentration of a) native β-CD and b) CD in the terpolymer. The spectra are normalized to the maximum intensity of the pure ANS solution.

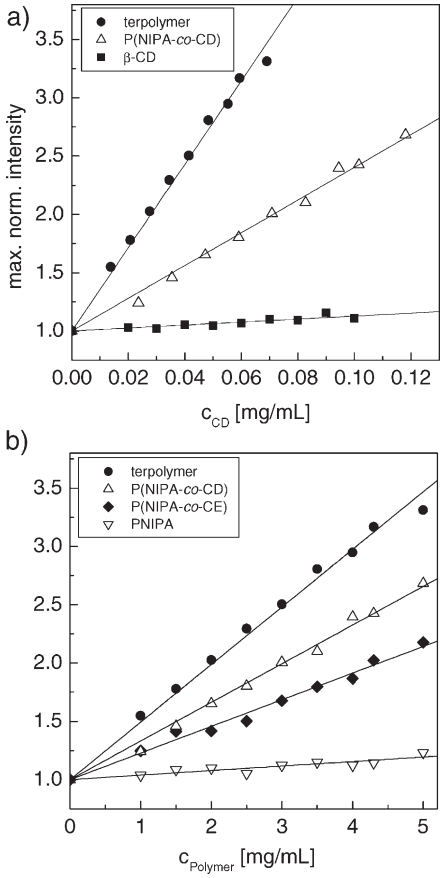


Figure 3.
Fluorescence intensity maxima in dependence of a) β-CD concentration (for polymers calculated by means of polymer composition), b) polymer concentration. The spetra are normalized to the maximum intensity of pure ANS solution.

stronger effect on the fluorescence of ANS than any of the polymers do.

However, the CE units in P(NIPA-*co*-CE) lead to an increased complexation, but to a lesser extend than P(NIPA-*co*-CD). Please note that the native CE has no effect on the ANS fluorescence intensity at all (not shown). So even without CD, the polymer-host molecule combination seems to shield ANS from water better than native β-CD is able to do.

An explanation for the shielding by the polymer coil may be the structure of the fluorescent dye ANS. It is a large, mostly aromatic compound and it will therefore preferably remain in a less polar environment instead of water. The polymer in combination with host molecules as CE and especially CD exhibit such additional interactions (CE complexes the counter ion and CD includes ANS itself) which causes the higher fluorescence intensities of ANS in presence of these copolymers. However, PNIPA alone does not seem to be able to shield ANS from water.

Conclusion

A significant enhancement in the fluorescence intensity of ANS in presence of the copolymer of CD and NIPA and the

terpolymer of CD, NIPA and CE could be observed compared to measurements in presence of native β-CD. As desired, the terpolymer shows the largest enhancement due to a synergistic cooperative interaction between the CE-counter ion complex and ANS included in the CD cavity.

The polymer chain plays an important role, too. This is reflected in fluorescence measurement of ANS in presence of P(NIPA-*co*-CE) and P(NIPA-*co*-CD) compared to measurements in presence of native β-CD.

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