Synthesis and Characterization of

Cholesterol Substituted Poly(*N*-isopropylacrylamides)

Synthesis, Characterization and Solution Properties of Cholesterol Substituted Poly(*N*-isopropylacrylamides)

By

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Abstract

Poly(N-isopropylacrylamides) and copolymers of N-(isopropylacrylamide) and N-(tetrahydrofurfurylacrylamide) bearing cholesterol substituents were prepared and their solution properties in water and in methanol were studied by dynamic light scattering, turbidity measurements and by fluorescence spectroscopy. These polymers were obtained in two steps: 1) preparation of a reactive polymer; and 2) coupling of cholesterol to the reactive polymer. In one case the reactive group (N-hydroxysuccinimide) was introduced at one chain end, in the other case, the same group was introduced randomly along the polymer chain. Reaction of an amino-terminated cholesteryl derivative with the reactive polymers led to modified polymers in which cholesterol was attached either to one chain end or to a small number of monomer units along the polymer chain. In the latter case, copolymers bearing pyrene and cholesterol were prepared as well. The copolymers were characterized by ^{1}H NMR, IR and UV spectroscopy and their molecular weights were determined.

Studies of the cholesterol-bearing polymers in aqueous solution by dynamic light scattering and fluorescence spectroscopy provided evidence for the formation of polymeric micelles via association of the cholesterol groups. Fluorescence probe and label experiments, using pyrene as a chromophore, gave strong indication that the micelle formation is controlled primarily by the cholesterol groups and only to a lesser extent by the architecture and composition of the polymer.

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List of Abbreviations

AIBN	2,2'-Azobis[2-methylpropionitrile]
Chol	cholesterol derivative
CAC	critical aggregation concentration
DCC	1,3-dicyclohexylcarbodiimide
DLS	dynamic light scattering
DMAP	*N, N*-dimethylamino-pyridine
HM	Hydrophobically-modified
IPA	Isopropylamine
IR	infrared spectroscopy
LCST	lower critical solution temperature
NASI	*N*-Acryloxysuccimide
NHS	*N*-Hydroxysuccinimide
NIPAM	*N*-Isopropylacrylamide
NMR	nuclear magnetic resonance
NTHFAM	*N*-Tetrafurfurylacrylamide
PNIPAM-COOH	oligomer NIPAM end-capped with a carboxylic group
PNIPAM/NTHFAM-COOH (PNT-COOH)	copolymer NIPAM/NTHFAM end-capped with a carboxylic group
PNIPAM-Chol	oligo NIPAM end-capped with cholesterol
PNIPAM/NTHFAM-Chol (PNT-Chol)	cooligo NIPAM/NTHFAM end-capped with cholesterol
PNIPAM	poly(*N*-isopropylacrylamide)
PNIPAM/NASI	copolymer NIPAM/NASI

PNIPAM/NTHFAM/NASI	copolymer NIPAM/NTHFAM/NASI
PNIPAM/Chol	PNIPAM randomly modified with cholesterol
PNIPAM/Py/Chol	PNIPAM randomly modified with pyrene and cholesterol
PNIPAM/NTHFAM/Chol (PNT/Chol)	PNIPAM/NTHFAM randomly modified with cholesterol
PNIPAM/NTHFAM/Py/Chol (PNT/Py/Chol)	PNIPAM/NTHFAM randomly modified with pyrene and cholesterol
Py	pyrene
TLC	thin layer chromatography
UV	ultraviolet spectroscopy

1. Introduction

Water soluble polymers represent a diverse class of polymers including biopolymers that mediate life processes as well as synthetic polymers, a large group of commercially useful products.[1-2] Synthetic water soluble polymers are used predominantly as viscosity and rheology modifiers,[1-10] and may replace naturally occurring polymers in technology and medicine.[11-12]

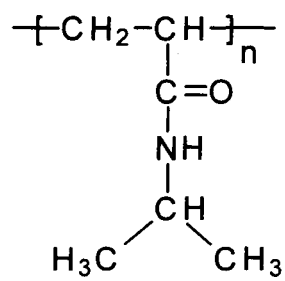
Water soluble polymers are classified as biopolymers, non-ionic, ionic (or polyelectrolytes) and associative polymers.[1, 12] Their solution properties are determined by the chemical structure of the polymer chain: the primary structure (the nature of the repeating units, composition, etc.) and the secondary structure (configuration, conformation and intramolecular effects such as hydrogen bonding, hydrophobic interactions and ionic interactions).[1]

The present work describes the study of a family of polyacrylamides. Hydrophobically modified poly(*N*-isopropylacrylamides) (HM-PNIPAM) and hydrophobically modified copolymers of *N*-Isopropylacrylamide (NIPAM) and *N*-Tetrahydrofurfurylamide (NTHFAM) were synthesized and characterized. Their solution properties were investigated by various physico-chemical techniques, including fluorescence.

1.1 Polymers and their solution properties in water

1.1.1 Poly(*N*-isopropylacrylamide) (PNIPAM)

Poly(*N*-isopropylacrylamide) (**PNIPAM, 1**), which belongs to the class of water soluble non-ionic polymers, has been extensively studied in several research groups, since it was first reported in 1956.[13-16] PNIPAM is soluble in cold water, however PNIPAM aqueous solutions undergo a reversible phase transition upon heating to a temperature of ca. 32 °C, known as the lower critical solution temperature (LCST).



(1)

(PNIPAM)

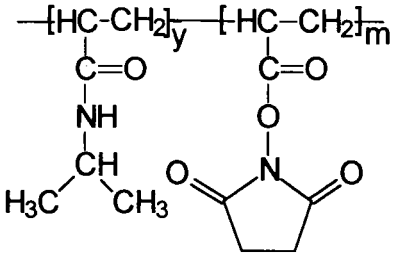
The LCST of PNIPAM in water was first reported by Heskins and Guillet[17]. It is believed that when a PNIPAM aqueous solution is heated above the LCST, PNIPAM chains are converted from a solvated-expanded coil to a collapsed globule. This transition results from the temperature-dependence of the hydrogen bonding between the polymer (the amide) and water molecules, the so called "hydrogen bonding effect" and of the hydrophobic interactions between polymer segments (the *N*-Isopropyl),[17] the so called

"hydrophobic effect". Fujishige *et al*[18] reported that the LCSTs of PNIPAMs in the range of molecular weight from 13.8 to 910 × 10^4 are independent of the molecular weight of polymers, while Schild and Tirrell[19, 20] observed a decrease in the LCSTs of PNIPAMs with increasing molecular weight of polymers (Mn from 5.3 × 10^3 to 1.6 × 10^5).

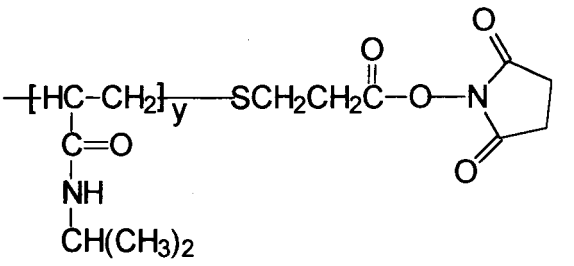
Free radical polymerization is the most common method to prepare PNIPAM. The most widely used initiators are azobisisobutyronitrile (AIBN) for organic media and $K_2S_2O_8$/ $Na_2S_2O_5$ (persulfate) for aqueous media. As in a typical free radical polymerization, the molecular weight distribution of PNIPAM is broad. The low conversion method has been reported to minimize the polydispersity (PD).[21]

1.1.2 Hydrophobically-modified PNIPAM (HM-PNIPAM)

The most significant advance in PNIPAM study during the past decade has been its modification with hydrophobic moieties. Hydrophobically-modified PNIPAMs (HM-PNIPAMs) have potential applications in areas such as viscosity modification, controlled drug release, and intelligent gel devices. HM-PNIPAMs are synthetic water soluble polymers that contain a small fraction of hydrophobic groups.[8, 22] The introduction of a small amount of hydrophobic groups on PNIPAM perturbs the hydrophobic-hydrophilic balance and can produce changes in the behavior of the polymer in water.[23]



2 (PNIPAM/NASI)



3 (Activated PNIPAM)

Usually, HM-PNIPAMs are prepared either by copolymerization of NIPAM and hydrophobic monomers[1, 8, 24, 25, 30] or by post-modification of reactive PNIPAM.[26-36] The first synthetic route is rather simple, compared to the second one. The second route, post modification of PNIPAM includes two options: one is the copolymerization of NIPAM and a monomer with an active group (2)[26, 28, 29] and the other is based on the use of a chain transfer agent [35, 36] during polymerization of NIPAM to cap the polymer chain with a functional end group (3). Post-modification of these polymers is performed by attaching hydrophobic molecules to the polymer chains through the active group or the functional end group. The resulting HM-PNIPAMs can be grouped into two types: (1) those with the hydrophobic pendant groups randomly distributed along chain; (2) those with a hydrophobic group attached to one end of the PNIPAM chain. Besides these commonly used synthetic routes, there are some alternative methods to prepare HM-PNIPAMs, for example, using a lipophilic initiator, 4, 4'-azobis(4-cyano-N, N'-

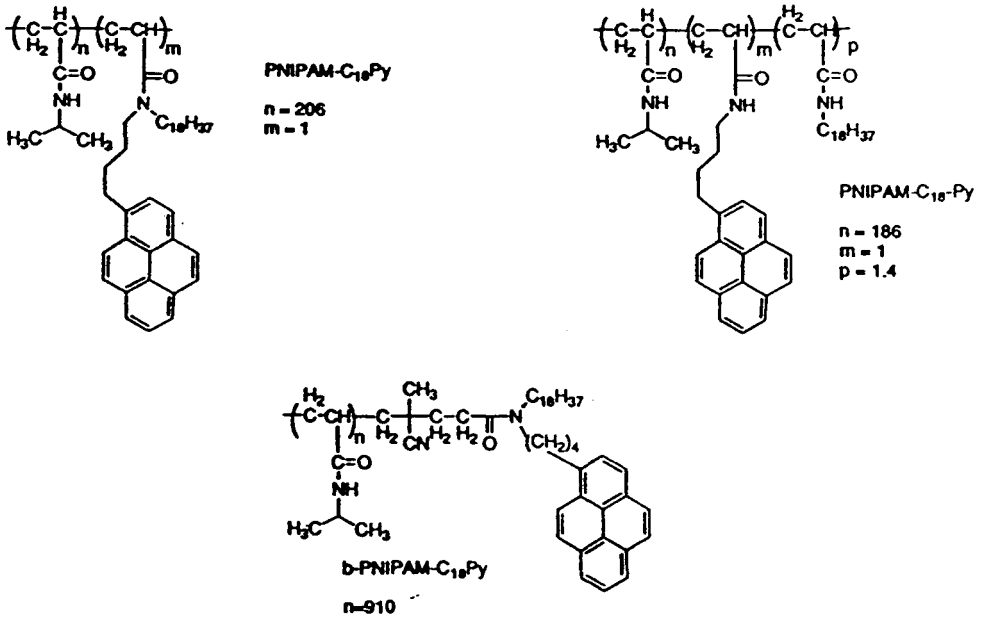
dioctadecyl) pentamide. Through free-radical polymerization, F. M. Winnik et al. prepared a HM-PNIPAM carrying two octadecyl groups at one chain end.[37]

HM-PNIPAMs, containing a small mole fraction of hydrophobic groups, aggregate in aqueous media forming hydrophobic microdomains. The polymer molecules are capable of forming interchain aggregates (micelles) in water and their behavior in aqueous solution is analogous to that of surfactant. The so-called micelles are relatively small spherical structures involving a few to a few thousand molecules arranged so that their hydrophobic regions aggregate in the interior, mostly excluding water, and their hydrophilic groups are at the surface in contact with water.[38-41] The randomly hydrophobically modified polymer will form intra- and inter- polymeric aggregates in their aqueous solution. At very low concentration, the hydrophobic interaction is limited to the same molecular chain, while at high concentration, both intra- and inter- molecular interactions exist. It is believed that the intermolecular aggregation of the hydrophobe is predominating.[42] In the case of end-capped HM-polymers, only inter- polymeric aggregates can form in aqueous solutions.

Schild and Tirrel reported the copolymerization of NIPAM with *N*-Hexadecylacrylamide (HDAAM) and discovered that 1.7 mol % HDAAM in PNIPAM copolymer led to insolubility of the polymer in water.[25] The LCSTs of the soluble copolymers (0.4 and 1.1 mol % HDAAM) were depressed by only ca. 2.5 °C, but the LCSTs' transition width of the endotherms obtained by DSC increased 3-4 times, in comparison with the behavior of the homopolymer. F.M. Winnik et al. prepared copolymers of NIPAM and n-alkylacrylamide (0.05 and 1 mol % copolymer, and akyl =

C_{10}, C_{14} and C_{18}) and found that below the LCST the aqueous solution properties of those modified PNIPAMs depend on the length of the alkyl group attached.[30] Fluorescence studies with fluorescence probes by both Schild and Tirrel [25] and Winnik[30] have given clear evidence of the formation of hydrophobic microdomains (micellar structures).

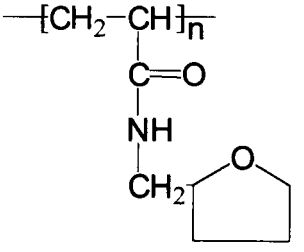
In order to study the relationship between the molecular architecture of HM-PNIPAM on its aggregation properties, F. M. Winnik *et al* also prepared PNIPAM with C_{18} linked in a different way on PNIPAM chain along with pyrene label.[34] The structures of those C_{18} modified PNIPAM are described in 4 (a), (b) and (c). Their fluorescence study provided information on the nature of the hydrophobic microdomains that form in these polymer solutions.



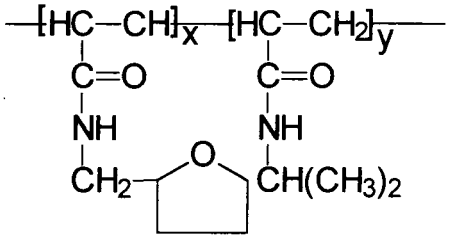
4 (a) PNIPAM-C_{18}Py; (b) PNIPAM-C_{18}-Py; (c) b-PNIPAM-C_{18}Py

1.1.3 The copolymer of NIPAM and NTHFAM

Poly(*N*-tetrahydrofurfurylamide) (**PNTHFAM, 5**) is an *N*-substituted poly alkoxylamide. Compared with NIPAM, the *N*-alkoxyalkyl substituent of NTHFAM is much more hydrophilic and can form more hydrogen bonds with water molecules.[43, 44] The polymer PNTHFAM was prepared previously and reported[43, 45-47] to have a high value of LCST (60-63 °C). It is a very hygroscopic polymer. These properties stem from the fact that *N*-Tetrahydrofurfuryl group contains an oxygen atom with an electron lone pair that is ready to form hydrogen bond with water molecules.[43,44]



5 (PNTHFAM)



6 (PNIPAM/PNTHFAM)

Copolymers of NIPAM and NTHFAM have not been reported. In this work we study both hydrophilic and hydrophobic effects on the aqueous solution of PNIPAM. NTHFAM (the structure of copolymer shown as **6**) was introduced on PNIPAM chain as the hydrophilic moieties. A few copolymers of PNIPAM/NTHFAM with different feed molar ratios of monomers were prepared by free radical polymerization. Their

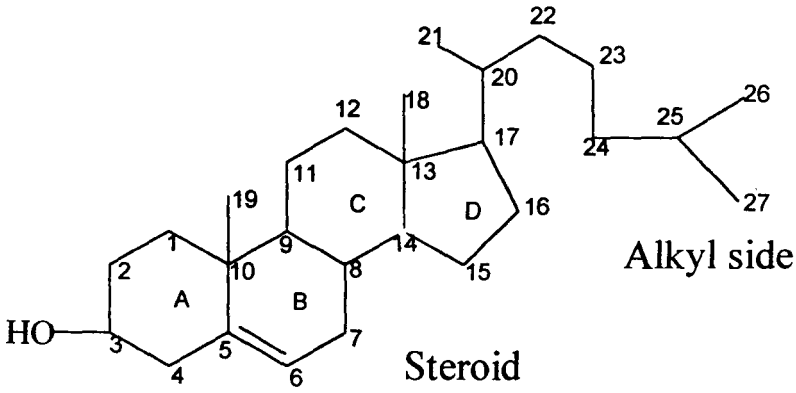
hydrophobically modified analogs were made by the same method as HM-PNIPAMs, utilizing either a monomer with an active group or a chain transfer agent. The free radical polymerization technique gives copolymers a random distribution of NIPAM and NTHFAM.

1.1.4 Cholesterol bearing polymers

1.1.4.1 Cholesterol: an integral part of human metabolism

Cholesterol, "the most highly decorated small molecule in biology",[48] is found in blood plasma and in all animal tissues. It is a white waxy solid and insoluble in water, as well as in blood, but it can be transported as a complex with a class of proteins (lipoprotein).[44] Cholesterol is not only an essential component of biological membranes, but also the parent steroid for the syntheses of sex hormones, adrenocorticoid hormones, bile acids and vitamin D.[38, 44, 49, 50]

The structure of cholesterol (**7**) and its stereochemistry (**8**) are shown below. Cholesterol, which is amphiphilic, has a secondary hydroxyl group at C_3 (the polar head), four fused hydrocarbon rings A, B, C, and D (the nonpolar hydrocarbon body) with a hydrocarbon side chain at C_{17} (nonpolar hydrocarbon tail).[49, 50] It spontaneously forms micelles, bilayers, and liposomes, stabilized by hydrophobic interactions.[38]



Alkyl side chain

Polar head

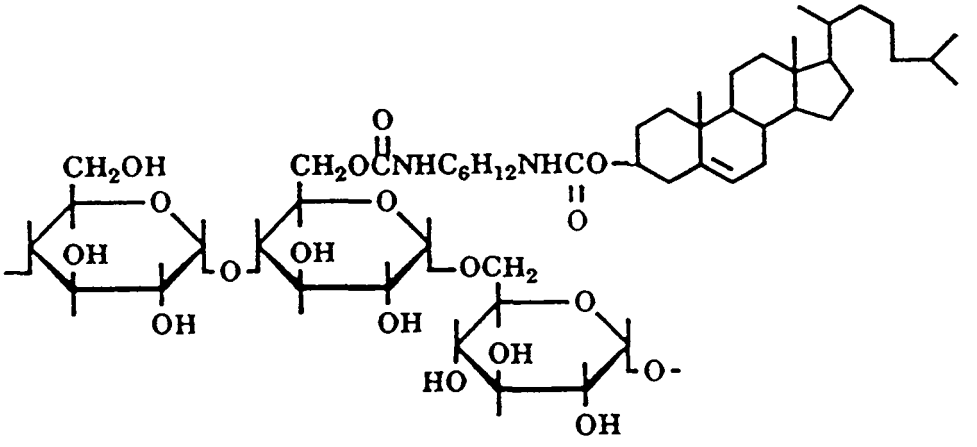
Steroid nucleus

7

8

1.1.4.2 The study of the cholesterol bearing polymers

The study on the self-organization and molecule recognition of lipids, proteins and nucleic acids is very important for understanding many biological phenomena and a variety of biological and medical applications.[51-55] In living systems, the association of various biopolymers is controlled by noncovalent bonding (secondary interactions). In a man-made system, however, it is not easy to control the polymer association through those noncovalent bondings.[51]



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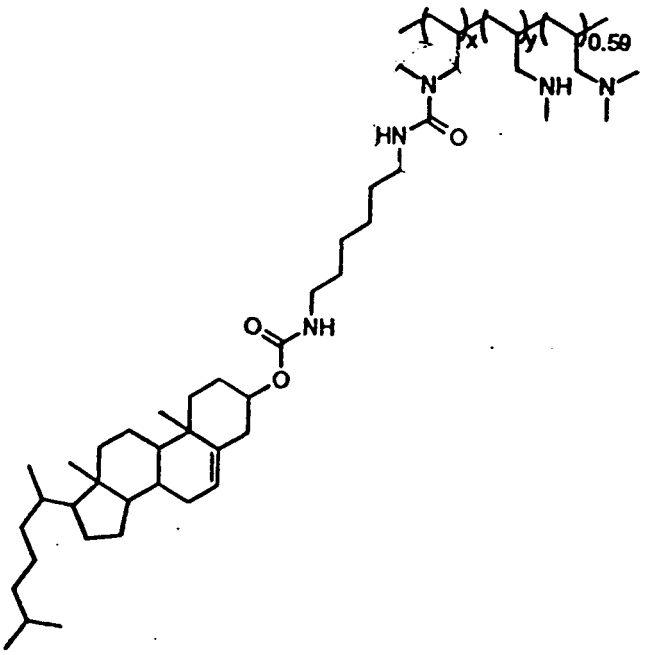
Sunamoto *et al* have studied cholesterol-bearing pullulan (Structure showed as **9**).[52-55] In aqueous solution the polymers form hydrogel nanoparticles by self-aggregation. The effects of the molecular weight of the pullulan and the amount of the cholesterol moiety on the microscopic structure of the nanoparticle hydrogel were studied extensively. It was clarified that the domains of the associated cholesterol groups provide cross-linking points by noncovalent bonding. The self-aggregates form complexes with

various hydrophobic substances and soluble proteins.[52,53] The hydrogel nanoparticle of cholesterol bearing self-aggregate was considered as a unique host for macromolecular guests such as proteins. It was suggested that the materials could be used in biotechnology and medicine.



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Tsuchida *et al*[51] studied the binding between cholesterol bearing poly (allylamine) (PAA, **10**) and bile salt by modifying poly (allylamine) with a cholesterol derivative (refer as cholesterol derivative 1 in this thesis). The study showed that the cholesterol-bearing polymer has excellent binding ability to self-aggregate and may be useful as a hypocholesterolemic agent under biological conditions.

It was shown that the solution properties of PNIPAM change with the introduction of n-alkyl groups.[30-37] Association of the hydrophobic groups affects on the polymer

conformation and eventually the rheological properties of the aqueous solution. It is attractive to investigate the solution properties of cholesterol bearing PNIPAM and gather information on possible applications in biotechnology and medicine. In the present work, cholesterol bearing PNIPAMs and PNIPAM/NTHFAMs were prepared for a basic study of the self-aggregation between the hydrophobically modified polymer chains (inter or intro) and water molecules.

1.2 Introduction to Fluorescence

When a species (an atom or a molecule) absorbs ultraviolet or visible radiation, it becomes excited. The lifetime of such energy rich states is rather short. The excited species rapidly lose the absorbed energy to return to the stable ground state. In general, several processes are responsible for the dissipation of the excess energy of an excited state in terms of photophysical and photochemical pathways.[56-59] Photochemical pathways are not discussed in this text.

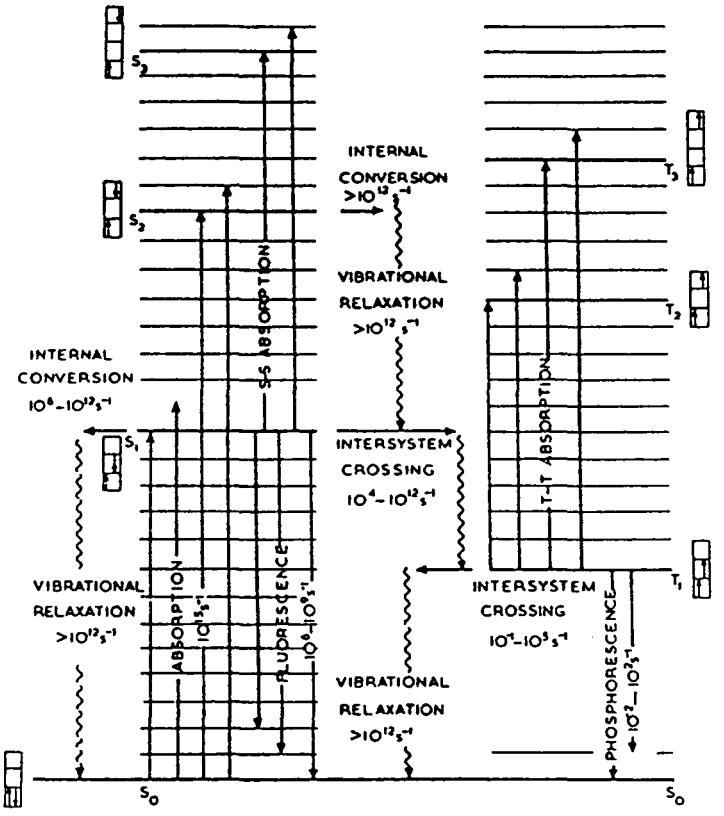


Figure 1.1 Jablonskii state diagram illustrating radiative and non-radiative decay paths of excited molecules

Photophysical processes are classified as radiative, when energy is lost by emission of radiation, and non-radiative, when there is no radiation during energy loss. Figure 1.1[58] presents a Jablonskii diagram which depicts the pathways of energy dissipation from the excited state. The radiation emitted during a radiative transition is called fluorescence when the transition is between states of the same multiplicity and phosphorescence when the transition is between states of different multiplicity, which means that fluorescence is $S_1 \rightarrow S_0$ Transition and phosphorescence is $T_1 \rightarrow S_0$

Transition. The present thesis deals with one of radiation (emission) in photophysical processes, ***fluorescence*** only.

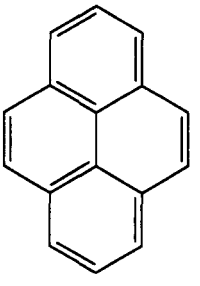
Fluorescence can arise by radiative emission from either (a) an S_1 state molecule or (b) an *excimer* formed through the association of an electronically excited molecule (S_1 state molecule) with an identical ground state molecule (S_0 state molecule).[56-62] If the two of chromophore molecules are far apart in their ground state (~ 10 Å), the excitation is localized on one of them, the excited molecule gives rise to normal fluorescence (monomer emission).[60, 62] The intermolecular interactions between the ground-state pair are very weak at large distance. The formation of excimer is due to the diffusive encounter between one ground state molecule and one excited molecule and depends on the distance between the pair. A certain orientation and distance between the two chromophore molecules are required for the formation of excimers,[58-62] since an chromophore molecule can only move a few angstroms in its life time ($\sim 10^{-9}$ sec for pyrene). There are two mechanisms for excimer formation. The excimer forms by excitation of a pre-associated chromophore pair, which is called "static excimer" and by diffusive encounter of one excited chromophore and one ground state chromophore, which is called "dynamic excimer".

(a) $S_1 \rightarrow S_0 + h\nu_f$ (normal or monomer fluorescence, $\mathbf{I_M}$)

(b) $S_1 + S_0 \rightarrow \underset{\text{Excimer}}{(S_1S_0)^*} \rightarrow 2S_0 + h\nu_{f(excimer)}$ (excimer fluorescence, $\mathbf{I_E}$)

Excimer was first observed by measuring the effect of increasing concentration on the solution fluorescence. The intensity of the normal fluorescence decreased, while a new broad and structureless band appeared at longer wavelengths (red-shifted). Pyrene (Py) shows this character strongly. Pyrene fluorescence is concentration-independent and gives pure monomer fluorescence at concentration of ca. 10^{-5} M or less. As Py concentration increases and the distances between the pyrene pairs is about 4 Å (close to the equilibrium separation of the excimer[60, 62]), the intensity of the monomer emission decreases and a new emission is observed at the red side of monomer emission. The dynamic nature of excimer formation can be confirmed by time-resolved experiments. For example,[60, 62] in a solution of pyrene (~10^{-3} M in cyclohexane) the excimer emission is growing with increase of excitation pulse.

Fluorescence emission is efficient in certain types of aromatic hydrocarbons. These aromatic hydrocarbons can be used to probe a variety of interesting characteristics of both small and large molecules. Pyrene (**11**) and its derivative 1-Pyrenemethylamine hydrochloride (**12**) are common probes and labels in polymer chemistry.



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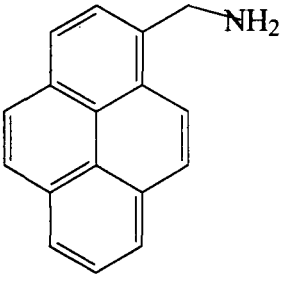

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1.3 The study of polymer association in water via fluorescence techniques

Light scattering, UV spectroscopy and fluorescence spectroscopy are common techniques for the study of the solution properties of PNIPAM and its modified derivatives. In this thesis, UV spectroscopy was used to determine the LCST of polymer aqueous solutions and Dynamic light scattering (DLS) was employed to gather information on the formation of micellar structure in HM-PNIPAM. The microstructure of polymer solution in water was studied via fluorescence techniques.

In the study of HM-polymers aqueous solution by fluorescence, there are two ways to apply dyes (chromophores). One method is to attach a dye derivative covalently on the polymer chain (we will use the word "label" here). Another is to employ the free dye as probe of the polymer aqueous solution (we will use the word "probe" here).[42, 60, 63]

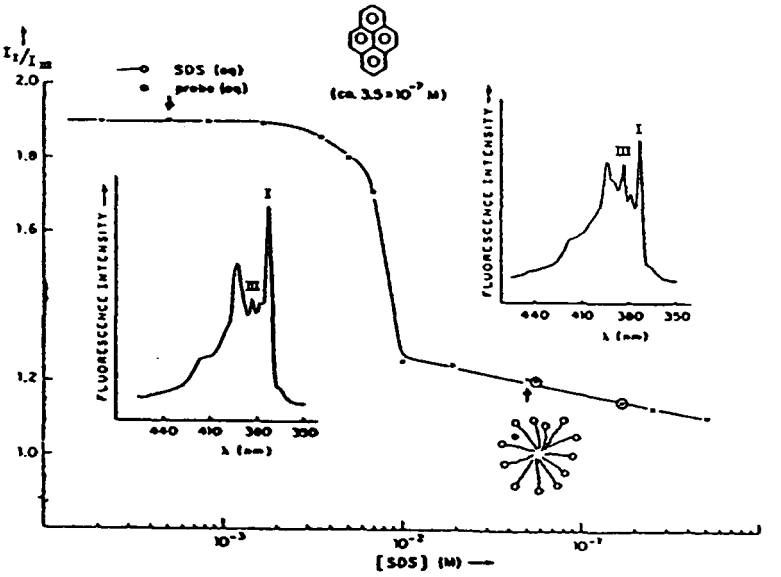
1.3.1 Pyrene probe in the study of micellar system

It has been over two decades since the vibronic band structure of pyrene fluorescence has been used to probe the micellar systems.[42, 61, 63-77] With this technique, one can get a measure of the effective polarity of that portion of the micelle where the probe is located.

The fine structure of pyrene fluorescence is quite dependent on solvent.[61, 66] In the case of pyrene, band III, (I_3 or I_{III} in Figure 1.2[69],) is not affected by solvent, but band I (0-0) (I_1 in Figure 1.2) is enhanced significantly with the increasing solvent polarity,[42, 61, 64-67, 69] For example, the I_1/I_3 ratio of pyrene is ca. 1.8 in water and 0.59 in dodecane.[66] Therefore, the ratio of intensity of I_1/I_3 is useful to monitor the changes in the

environment of a pyrene probe. Pyrene is very hydrophobic and has a very low solubility in water (ca. 7×10^{-7} M). In the presence of micelles, pyrene is preferentially solubilized in or near the interior hydrophobic regions of these aggregates.[42, 61, 64, 65, 69]

Studied by Turro *et al,* Figure 1.2 [69] is an example of the use of pyrene as a fluorescence probe for micelle formation by sodium dodecyl sulfate (SDS), an anionic surfactant. The ratio I_1/I_3 at very low concentration of surfactant is close to that in water and indicates that the probe is in the water phase. At concentration of SDS over 10^{-2} M, the ratio I_1/I_3 is ca. 1.1, indicating that pyrene is now solubilized in the hydrophobic regions of the aqueous solution.



. **Demonstration of the use of pyrene as a fluorescence probe for micelle formation by SDS in aqueous solution. The points on the line correspond to the I_1/I_{III} values (see text for discussion) as a function of SDS concentration. The inserts are spectra of pyrene fluorescence below the cmc (concentration indicated by heavy arrow) and above the cmc (concentration indicated by heavy arrow).**

Figure 1.2 The fine structure of pyrene fluorescence in water and in the micellar system (SDS)

The properties of hydrophobically modified polymers in aqueous solution can also be investigated by using the I_1/I_3 parameter. The CAC (critical aggregation concentration) of hydrophobically modified polymers can be obtained by the study of I_1/I_3 as a function of polymer concentration. Winnik *et al* studied the HM-PNIPAM by fluorescence with different techniques[30-37] and using pryrene as probe is one of the successful examples. For instance, Figure 1.3 is a plot of the changes in the ratio of I_1/I_3 for pyrene in aqueous solutions of alkyl chain bearing PNIPAM as a function of alkyl chain concentration in polymers. The results confirmed the existence of polymeric micelles below the polymers' LCST in C_{14} and C_{18} bearing PNIPAMs' aqueous solutions but not in C_{10} bearing PNIPAM.[30]

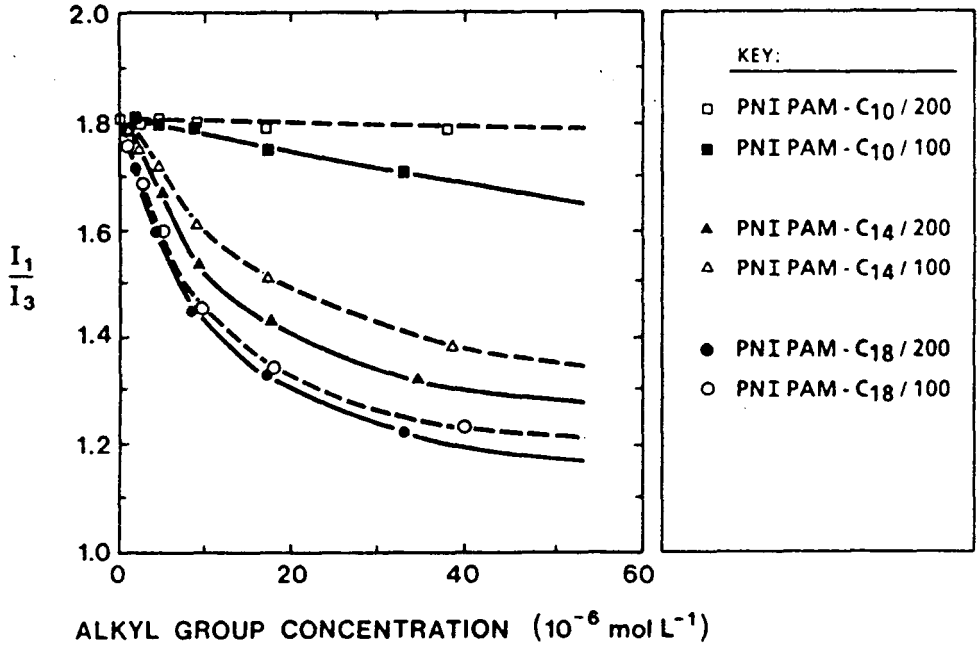


Figure 1.3 I_1/I_3 as a function of alkyl concentration in alkyl bearing PNIPAMs (15°C)

1.3.2 Attaching a pyrene derivative to polymer chain

A pyrene-labeled PNIPAMs containing both pyrene and hydrophobic group (C_{18} group) randomly attached on the same chain is shown as **4** in section **1.1.2**. The monomer emission I_M and the excimer emission I_E are observed at wavelengths lower than 400 nm and ca. 480 nm, respectively. The ratio I_E/I_M is commonly used as a measure of excimer formation.[42, 60] In a micellar system, the essential prerequisite to excimer formation is that the micelle has to contain at least two pyrene molecules.[42, 60, 61] If a micelle contains only one pyrene molecule, excimer formation does not occur since there is not enough time for a second pyrene molecule to contact the excited pyrene from one micelle to another during the life time of this excited pyrene. We must note that in macromolecules only certain conformations of the flexible chain will be favorable to excimer formation,[59, 61] and in order to exhibit excimer emission, the conformations must be reached in appreciable quantities within the lifetime of the excited state of the excited pyrene. In other words, information of the conformation of molecules in solution can be obtained by measuring the ratio I_E/I_M.

In a pyrene labeled HM-polymer solution, though the content of pyrene label is very low, if the polymer chain provides the proper conformation, pyrene excimer can form either intramolecularly and/or between several polymer chains. By monitoring the changes in I_E/I_M as a function of polymer concentration, one can determine the CAC (critical aggregation concentration) of the hydrophobically modified polymer and distinguish the occurrence of the intra- and intermolecular interaction.[28]

The excitation spectra of the labeled polymer monitored at monomer emission (ca.380 nm) and at excimer emission (ca. 480 nm) provide information on the mechanism (static excimer or dynamic excimer) of pyrene excimer formation. The parameters investigated are described in Table 1.1. and an example of excitation spectra monitored for the monomer (379 nm) and excimer (489 nm) emission are shown in Figure 1.4 (an HM-polymer solution). Both spectra have similar shapes but are very different in the positions of the band maxima and in the widths of band, when the preassociation of pyrene labels on polymer chains occurs before the excitation due to the hydrophobic interactions. The red-shifts ($\Delta\lambda = \lambda_{max,\ excimer}$ (monitored at 480nm)- $\lambda_{max,\ monomer}$ (monitored at 380nm) is mildly dependent on the level of pyrene attached and can give a indication on the extent of pyrene aggregation in aqueous solution.[60]

Table 1.1 The parameters are being investigated in excitation spectrum[60]

Parameters	Definitions
P_M	Peak to valley ratio of [0,0] transition excited at ca. 380 nm (monomer emission)
P_E	Peak to valley ratio of [0,0] transition excited at ca. 480 nm (excimer emission)
$\Delta\lambda$, nm	The difference of wavelength maxima of [0,0] transition in excimer and monomer spectra

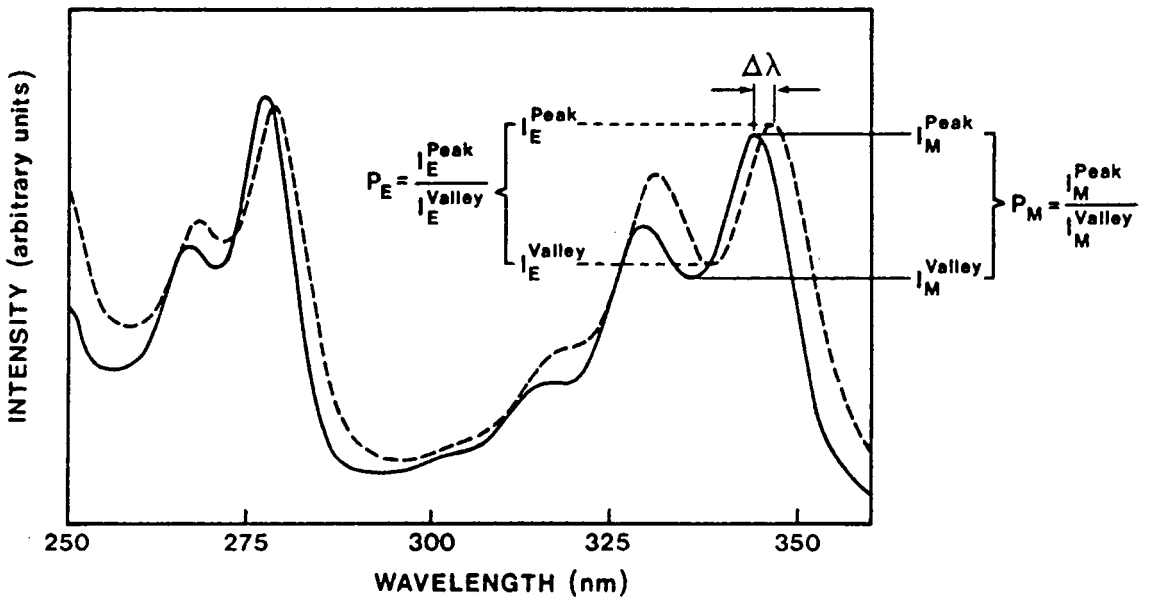


Figure 1.4 Normalized excitation spectra of HM-polymer in water the solid line monitored at 380 nm, monomer emission; the dashed line monitored at 489 nm, excimer emission.

1.4 The Objectives

The objectives of this work are two folds. The first objective is to prepare and characterize cholesterol bearing oligomers and polymers, which include functionalization of cholesterol (the preparation and characterization of cholesterol derivatives), the synthesis and characterization of the monomer (NTHFAM) and the synthesis and characterization of cholesterol bearing polymers. The second objective is to study the solution properties of the cholesterol bearing polymers. UV spectroscopy was used to determine the LCSTs of the polymers in aqueous solution. Dynamic light scattering measurement and fluorescence spectroscopy through both label and probe techniques, were performed to collect evidence for the formation of polymeric micelles and to characterize the solution properties of cholesterol bearing NIPAM polymer and copolymers.

2. Experimental Section

2.1 Materials

Cholesterol (5-Cholesten-3β-ol, $C_{27}H_{46}O$, 99+%) was obtained from Sigma. 1,6-Hexyldiisocyanate (97%) was purchased from Aldrich Chemicals. Ethylenediamine and pyridine were purchased from Fisher Scientific and used without purification.

Tetrahydrofurfurylamine, acryloyl chloride and 2,6-di-*tert*-butyl-4-methyl-phenol (99%) were purchased from Aldrich Chemicals.

N-Isopropylacrylamide (NIPAM, from Eastman Kodak Company) was purified twice by recrystallization from toluene/hexane (1/1 v/v). Mercaptopropionic acid (99%), *N*-hydroxysuccinimide (NHS) (97%), 1-pyrenemethylamine hydrochloride (95 %), 1,3-dicyclohexylcarbodiimide (DCC) (99%), and 4-dimethylaminopyridine (DMAP) (99+%) were obtained from Aldrich. Isopropylamine was also purchased from Aldrich and was distilled before use. Pyrene (99+ %, from Aldrich) was recrystallized twice from methanol and followed by sublimation before use. *N*-Acryloxysuccinimide (NASI) was obtained from Acros Chemicals. Triethylamine from Fisher Scientific was distilled before use. 2,2'-Azobis[2-methylpropionitrile] (AIBN) was purchased from Spectrum Quality Products, Inc. and was used without further purification.

Water was purified using a NANOpure deionizing system. All solvents used were reagent grade and were used as received except that tetrahydrofuran (THF), toluene

and 1,4-dioxane were dried over molecular sieve type 4A for at least 48 hours before use. Deuterated chloroform (99.8%) was purchased from Cambridge Isotope Laboratories.

Thin layer chromatography (TLC) was performed on silica plates (Merck) eluted with $CHCl_3$ or CHCl3 / MeOH (9/1 v/v). All the polymerizations were carried out under a nitrogen atmosphere.

2.2 Instrumentation

^1H NMR spectra were recorded on a Brucker AC 200 or 500 MHz spectrometer. IR spectra were obtained using a Bio-Rad FTS-40 FTIR spectrometer with samples prepared as KBr pellets. Mass spectrometry was performed on a FINNIGAN 4500 mass spectrometer using either chemical ionization (CI). UV spectra were measured on a Hewlett-Packard 8452 Photodiode Array spectrophotometer, equipped with a Hewlett-Packard 89090A temperature controller, and operated through HP ChemStation Windows-based software.

Potentiometric titration was performed with a Tanager Scientific Systems (model 8901, Dual pH meter and titrimeter) equipped with TANA 58-2 software.

Melting points were recorded on a Gallenkamp Melting Point Apparatus (Registered Design No. 889339) with a closed end capillary (size 1.5-1.8 x 90 mm).

Molecular weights and molecular weight distributions of the polymers were determined by gel permeation chromatography (GPC) on a GPC system consisting of a Waters 590 programmable pump equipped with four PL columns and a Waters 410

differential refractometer with THF as the mobile phase and a set of narrow dispersed polystyrene as standard for calibration.

Dynamic Light Scattering measurements were performed on a Brookhaven Instrument Corp. Model BI9000AT correlator equipped with a Lexel Argon laser (λ=514 nm, scattering angle = 90 degree) and the temperature was set at 25 °C.

Fluorescence spectra were recorded on a SPEX Fluorolog 212 spectrometer equipped with a DM3000F data system. The instrument was equipped with a water-jacketed cell holder controlled by a Neslab circulating bath. The slit widths were set at 0.7 or 1.0 mm (emission) and 2.0 or 1.0 mm (excitation) depending on the concentration of chromophore.

Solvent evaporation was done by a Rotary Evaporator RE 47 equipped with a B-169 vacuum system (Büchi). Aqueous polymer samples were freeze-dried by a Virtis Sentry™ Benchtop 3L Lyophilizer.

2.3 Methods

2.3.1 Syntheses of cholesterol derivatives

2.3.1.1 Synthesis of cholesteryl *N*-(6-isocyanatohexyl)carbamate (cholesterol derivative 1)

1,6-Hexyldiisocyanate (48 mL, 0.30 mol), 5-cholesten-3β-ol (7.8 g, 0.020 mol) and pyridine (4 mL, 0.049 mol) were dissolved in dry toluene (200 mL) in a 500-mL three-necked round bottom reaction flask equipped with a heating mantle, a magnetic stirrer, reflux condenser and a thermometer. The reaction mixture was stirred reflux at 80 °C for 48 h. The progress of the reaction was followed by TLC. The solvent and the excess 1,6-hexyldiisocyanate were removed by vacuum distillation (ca. 135°C, 0.3 mm Hg). Petroleum ether (600 mL) was added to the remaining solution and it was kept at -12°C for over 48 hours for crystallization. The precipitate was filtered through a Buchner funnel and dried *in vacuo* to give cholesterol derivative 1 as a white powder (7.0 g, 63 %). ^{1}H NMR (500 MHz, $CDCl_3$ with TMS): δ (ppm) = 0.65 (s, 3H, cholesterol $3H_{18}$), 0.84 (d, 6H, J=6.57 Hz, cholesterol $3H_{26}$ and $3H_{27}$), 0.89 (d, 3H, J=6.47 Hz, cholesterol $3H_{21}$), 0.98 (s, 3H, cholesterol $3H_{19}$), 1.40-1.50 (m, 8H, $[C\underline{H}_2]_4$), 0.98-2.40 (m, 28H, cholesterol $2H_1$, $2H_2$, $2H_4$, $2H_7$, H_8, H_9, $2H_{11}$, $2H_{12}$, H_{14}, $2H_{15}$, $2H_{16}$, H_{17}, H_{20}, $2H_{22}$, $2H_{23}$, $2H_{24}$, H_{25}), 3.14 (d, 2H, J=6.09 Hz, HN-$C\underline{H}_2$), 3.27 (t, 2H, J=6.65 Hz, $C\underline{H}_2$NCO), 4.4 (s, 1H, cholesterol, H_3), 4.6 (s, 1H, N$\underline{H}$), 5.35 (s, 1H, cholesterol H_6). Mass ($M+H^+$, %): 147 (15), 161 (8), 368 (100), 384 (7), 555 (7). IR (cm^{-1}, KBr pellet): 3345 (N-H), 2254 (N=C=O), 1692 (C=O), 1037 (O-C=O). M.P (°C, 760 mm Hg): 93-98.

2.3.1.2 Synthesis of cholesterol derivative 2

Ethylene diamine (2 mL, 3 mmol) with 1 mL of pyridine was dissolved in ether (10 mL). The cholesterol derivative 1 (0.48 g, 0.09 mmol) in ether (15 mL) was added to the solution slowly over 5 min. at room temperature. A white precipitate was observed immediately and accumulated during the addition of cholesterol derivative 1. The reaction then was kept for another 2.5 h at room temperature. The reaction was monitored by TLC. The solution was placed at -10°C for 0.5 hour. The white solid was collected by vacuum filtration and washed with cold ethyl ether. The final product was a white solid (5.0 g, 82 %). ^{1}H NMR (500 MHz, $CDCl_3$ with TMS): δ (ppm) = 0.66 (s, 3H, cholesterol $3H_{18}$), 0.85 (q, 6H, J=6.57 Hz, cholesterol $3H_{26}$ and $3H_{27}$), 0.89 (d, 3H, J=6.47 Hz, cholesterol $3H_{21}$), 0.99 (s, 3H, cholesterol $3H_{19}$), 130-1.60 (m, 8H, $[C\underline{H}_2]_4$), 1.23 (s, 2H, $N\underline{H}_2$), 0.98-2.40 (m, 28H, cholesterol $2H_1$, $2H_2$, $2H_4$, $2H_7$, H_8, H_9, $2H_{11}$, $2H_{12}$, H_{14}, $2H_{15}$, $2H_{16}$, H_{17}, H_{20}, $2H_{22}$, $2H_{23}$, $2H_{24}$, H_{25}), 2.82 (s, 2H, $H_2NC\underline{H}_2CH_2$), 3.14 (s, 2H, $H_2NCH_2C\underline{H}_2$), 3.15 (s, 2H, $C\underline{H}_2$NHCOO-cholesterol), 3.22 (t, 2H, J=4.91 Hz, $NHC\underline{H}_2C_5H_{10}$), 4.5 (s, 1H, cholesterol, H_3), 4.66 (s, 1H, N<u>H</u>COO-cholesterol), 4.85 (s, 1H, NHCON<u>H</u>), 4.91 (s, 1H, $NH_2CH_2CH_2N\underline{H}$), 5.34 (s, 1H, cholesterol H_6). Mass ($M+H^+$, %): 247 (10), 615.7 (100), 714 (22). IR (cm^{-1}, KBr pellet): 3344 (N-H), 3200 (N-H), 1689 (C=O), 1583 (CNH), 1037 (O-C=O).

2.3.2 Synthesis of *N*-Tetrahydrofurfurylacrylamide (NTHFAM)

Tetrahydrofurfurylamine (48 mL, 460 mmol) with a pinch of 2,6-di-tert-butyl-4-methylphenol in pre-cooled (-10° C) toluene (50 mL) was charged into a 500-ml three-necked round bottom flask equipped with a magnetic stirrer, reflux condenser, ice bath and nitrogen inlet. The solution was stirred and degassed by bubbling N_2 for 20 min. Triethylamine (60 mL, 430 mmol) dissolved in pre-cooled toluene (100 mL) was added and followed by the slow addition of acryloyl chloride (30 mL, 369 mmol) dissolved in pre-cooled toluene (200 mL). The solution was allowed to stir at room temperature for 20 h. The triethylamine hydrochloride was filtered from the solution and toluene was removed *in vacuo*. Vacuum distillation (145°C, 0.3 mm Hg) in the presence of 2,6-di-tert-butyl-4-methylphenol gave the monomer THFAM as a yellowish oily liquid (35 g, 54.7 %). ^{1}H NMR (200 MHz, $CDCl_3$ with TMS): δ (ppm) = 1.52-2.05 (m, 4H, THFAM $4H_b$), 3.16-3.69 (m, 2H, THFAM 2Hc), 3.70-4.04 (m, 3H, THFAM $3H_d$), 5.60-5.66 (dd, 1H, $H_bC\underline{H}_a{=}CH_c$, J_{ac}= 10.3 Hz, J_{ab}=1.5 Hz), 6.07 (dd, 1H, $H_bCH_a{=}C\underline{H}_c$, J_{bc}=17.0 Hz, J_{ac}=10.3 Hz), 6.55 (dd, 1H, $\underline{H}_bCHa{=}CH_c$, J_{ac}=17.0 Hz, J_{ab}=1.5 Hz), 7.34 (s, 1H, CON$\underline{H}$).

2.3.3 Syntheses of copolymers

All polymers in this research were prepared by free radical polymerization.

2.3.3.1. Reactive copolymer PNIPAM/NASI (molar ratio: 100/2)

A solution of NIPAM (12.5 g, 110.6 mmol) and NASI (0.372 g, 2.20 mmol) in dry 1,4-dioxane (240 mL) was stirred at room temperature under a nitrogen atmosphere

for 20 min. before being placed into a 65°C-oil bath. AIBN (91 mg, 0.55 mmol) in dry 1,4-dioxane (15 mL) was added into solution. The reaction mixture was allowed to stir for 18 h at 65 °C under a nitrogen atmosphere. The excess solvent was removed by rotary evaporation. The polymer was recovered by precipitation in hexane (2 L). It was purified further twice by precipitation from THF (~ 30 mL) into diethyl ether (2 L) and dried *in vacuo* (9.1 g, 70 %). IR (cm^{-1}, KBr): 3440, 3313, 2975, 2937, 1820, 1783, 1741, 1716, 1651, 1547.

2.3.3.2 Reactive copolymer PNIPAM/NTHFAM/NASI (molar ratio: 70/30/2)

This copolymer (13 g, 85 %) was prepared by the same procedure as described in **2.3.3.1**, starting with NIPAM (9.2 g, 81.4 mmol), NTHFAM (5.5 g, 35 mmol), NASI (0.392 g, 2.30 mmol) and AIBN (96.6 mg, 0.58 mmol) in dry 1,4-dioxane (350 mL). IR (cm^{-1}, KBr pellet): 3440, 3313, 2974, 2936, 1808, 1783, 1741, 1712, 1653, 1546, 1075.

2.3.3.3 Oligomer PNIPAM terminated with carboxylic group (PNIPAM-COOH)

The molar ratio of NIPAM (monomer), AIBN (initiator) and mercaptopropionic acid (chain transfer agent) to prepare this oligomer was 100:1:2.2. A solution of NIPAM (3.2 g, 28.3 mmol) and mercaptopropionic acid (66 mg, 0.62 mmol) in MeOH (40 mL) was stirred at room temperature and degassed by bubbling N_2 for 20 min. before being placed into a 65°C-oil bath. AIBN (46.5 mg, 0.283 mmol) was added to solution. The solution was allowed to stir for 18 h at 65°C under a nitrogen atmosphere. The excess MeOH in solution was evaporated *in vacuo* and the polymer was precipitated in ethyl

ether (1.0 L). It was purified further by two additional precipitations from THF (~ 15 mL) into diethyl ether (1.0 L) and dried *in vacuo* (2.4 g, 75 %). Mn (acid/base titration) ~2,000. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) = 1.13 (s, 6H, NCHC$\underline{H}_3$), 1.30-3.0 (m, polymer backbone C$\underline{H}_2$), 3.99 (broad s, 1H, NC$\underline{H}$), 6.4 (s, 1H, N$\underline{H}$).

2.3.3.4 Oligomer PNIPAM/NTHFAM terminated with carboxylic group (PNIPAM/NTHFAM-COOH or PNT-COOH)

The molar ratio of NIPAM (monomer), THFAM (monomer), AIBN (initiator) and mercaptopropionic acid (chain transfer agent) to prepare this oligomer was 70:30:1:2.2. The oligomer was prepared by following the same procedure as described in section **2.3.3.2**, however, two monomers, NIPAM (10 g, 88.4 mmol) and NTHFAM (5.9 g, 37.9 mmol) with the molar ratio of 70/30 were used. The excess MeOH in solution was evaporated *in vacuo* and the oligomer was precipitated in ethyl ether (2.0 L). It was purified further by two additional precipitation from THF (~ 30 mL) into diethyl ether (2.0 L) and dried *in vacuo* (11.8 g, 74 %). Mn (acid/base titration) ~ 1,950.

2.3.4 Modification of the polymers

2.3.4.1 Modification of PNIPAM/NASI with the cholesterol derivative 2 (PNIPAM/Chol)

PNIPAM/NASI (from section 2.3.3.1, 0.5 g, NASI ~ 0.088 mmol), the cholesterol derivative 2 (65 mg, 0.11 mmol) and triethylamine (14 mg, 0. 14 mmol) were dissolved in THF (15 mL). The solution was stirred under constant N_2 at room temperature for two

days. The unreacted succinimide group was quenched with isopropylamine. The polymer was precipitated in 520 mL of hexane/toluene (v/v: 50/2). It was purified further twice by precipitation from THF (~ 8 mL) into 520 mL of hexane/toluene (v/v: 50/2) and once from THF to diethyl ether, then dried *in vacuo*. The dry polymer was dissolved in water. The solution was filtered through NALGENE filter (0.45 μm). The polymer was recovered by lyophilization of the solution (0.28 g, 56 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) = 0.66 (s, 3H, cholesterol $3H_{18}$), 0.84-5.35 (m, cholesterol), 1.12 (s, 6H, NCHC$\underline{H}_3$), 1.33-2.75 (m, polymer backbone C$\underline{H}_2$), 3.98 (broad s, 1H, NC$\underline{H}$), 6.4 (s, 1H, N$\underline{H}$). IR (cm^{-1}, KBr pellet): 3426, 3311, 3076, 2972, 2936, 2874, 1648, 1548, 1462, 1387, 1368, 1250, 1173, 1131, 672.

2.3.4.2 Modification of PNIPAM/NASI with the cholesterol derivative 2 and 1-pyrenemethyl amine hydrochloride (PNIPAM/Py/Chol)

The same procedure and the same copolymer (from **2.3.3.1**) as described in section **2.3.4.1** were used to prepare this polymer, by using the cholesterol derivative 2 and the pyrene derivative instead of cholesterol derivative 2 alone. PNIPAM/NASI (from section 2.3.3.1, 2.0 g, NASI 0.342 mmol) was dissolved in THF (23 mL). 1-Pyrenemethylamine hydrochloride (45 mg, 0.17 mmol) and triethylamine (20 mg, 19.8 mmol) were added at once. The solution was kept in the dark and stirred for 24 h at room temperature. The cholesterol derivative 2 (125 mg, 0.21 mmol) in toluene/DMSO (22 mL, 5:6 v/v) was added to the reaction solution. The mixture was stirred at room temperature for an additional three-day period. The unreacted succinimide group was

quenched with isopropylamine. The polymer was precipitated in hexane (700 mL). It was purified by MeOH in ethyl ether once, THF/toluene in hexane/toluene (500/ 20 mL) twice and THF in ethyl ether once before being dried *in vacuo* (1.5g, 75 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) = 0.65 (s, 3H, cholesterol $3H_{18}$), 0.84-5.33 (m, cholesterol), 1.12 (s, 6H, NCHC$\underline{H}_3$), 1.33-2.75 (m, polymer backbone C$\underline{H}_2$), 3.97 (broad s, 1H, NC$\underline{H}$), 6.4 (s, 1H, N$\underline{H}$), 7.9-8.7 (m, protons from PY). UV (wavelength, nm) λ = 212, 244, 266, 278, 328, 344.

2.3.4.3 Modification of PNIPAM/NTHFAM/NASI with the cholesterol derivative 2 (PNIPAM/NTHFAM/Chol or PNT/Chol)

The modification of this polymer was performed using the same method as described in section 2.3.4.1, starting with PNIPAM/NTHFAM (2.0 g, 0.320 mmol of NASI) and the cholesterol derivative 2 (0.23 g, 0.38 mmol). The polymer was reprecipitated twice from THF/toluene into ether/toluene (9/1, v/v) and once from THF into ether and dried *in vacuo* (1.0 g, 50 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) = 0.65 (s, 3H, cholesterol $3H_{18}$), 0.84-5.35 (m, cholesterol), 1.11 (s, 6H, NCHC$\underline{H}_3$), 1.30-3.90 (m, polymer backbone C$\underline{H}_2$ and protons from THFAM), 3.96 (broad s, 1H, NC$\underline{H}$), 6.7 (s, 1H, N$\underline{H}$). IR (cm^{-1}, KBr): 3439, 3307, 3081, 2975, 2938, 2877, 1653, 1550, 1460, 1388, 1369, 1272, 1132, 1075, 926, 667.

2.3.4.4 Modification of PNIPAM/NTHFAM/NASI with the cholesterol derivative 2 and 1-Pyrenemethylamine hydrochloride (PNIPAM/NTHFAM/Py/Chol or PNT/Py/Chol)

The preparation of this polymer followed the method described in section 2.3.4.2 starting with PNIPAM/NTHFAM/NASI (2.1 g, 0.334 mmol of NASI), 1-Pyrenemethylamine hydrochloride (0.045 g, 0.17 mmol), triethylamine (0.06 g, 0.59 mmol) and cholesterol derivative 2 (0.125 g, 0.20 mmol). The polymer was recovered *in vacuo* (1.2 g, 57%). ^{1}H NMR (500 Hz, $CDCl_3$) δ (ppm) = 0.65 (s, 3H, cholesterol $3H_{18}$), 0.84-5.35 (m, cholesterol), 1.11 (broad s, 6H, NCHC$\underline{H}_3$), 1.30-3.90 (m, polymer backbone C$\underline{H}_2$ and protons from THFAM), 3.96 (broad s, 1H, NC$\underline{H}$), 6.7 (s, 1H, N$\underline{H}$), 7.9-8.5 (m, protons from PY). UV (wavelength, nm) λ = 208, 236, 244, 266, 278, 328, 344.

2.3.4.5 Modification of PNIPAM-COOH (oligomer) with *N*-Hydroxysuccinimide and the cholesterol derivative 2 (PNIPAM-Chol)

2.3.4.5.1 Activated PNIPAM-COOH

PNIPAM-COOH (2.4 g, 1.2 mmol of COOH, from section **2.3.3.3**), *N*-Hydroxysuccinimide (0.26 g, 2.4 mmol), DCC (1.0 g, 4.8 mmol) and DMAP (0.15 g, 1.2 mmol) were dissolved in dry THF (50 mL). The solution was stirred at room temperature under N_2 for 18 hours. The solution was filtered twice to remove the precipitate. The excess THF in the filtrate was removed by rotary evaporator. The oligomer was precipitated in ethyl ether (1 L). It was purified further by an addition precipitation from

THF (~ 15 mL) into diethyl ether (1 L) and dried *in vacuo* (2.0 g, 80 %). IR (cm^{-1}, KBr pellet): 3436, 3299, 2975, 2937, 1816, 1785, 1739, 1651,1546.

2.3.4.5.2 Modification of the activated oligomer with the cholesterol derivative 2

The aforementioned activated oligomer in section **2.3.4.5.1** (1.4 g, and 0.7 mmol) was dissolved in dry THF (70 mL). The cholesterol derivative 2 (0.47 g, 0.77 mmol) in toluene/DMSO (200 mL, v/v: 1/1) was added to the solution in 10 % molar excess, relative to the end group of the polymer. The solution was stirred at room temperature for 18 h. The proceeding of the reaction was monitored by TLC. The unreacted succinimide group was quenched with isopropylamine. The oligomer was precipitated in ether (500 mL) after removing the excess solvent. It was purified further by two precipitations from THF (~ 8 mL) into ether (500 mL), then dried *in vacuo* (0.5 g, ~ 30%). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) = 0.66 (s, 3H, cholesterol $3H_{18}$), 0.84-5.34 (m, cholesterol), 1.12 (s, 6H, NCHC$\underline{H}_3$), 1.31-2.75 (m, polymer backbone C$\underline{H}_2$), 3.99 (broad s, 1H, NC$\underline{H}$), 6.2 (s, 1H, N$\underline{H}$). IR (cm^{-1}, KBr pellet): 3308, 3078, 2974, 2936, 2876, 1651, 1547, 1462, 1388, 1364, 1264, 1174, 1132, 665.

2.3.4.6 Modification of PNIPAM/NTHFAM-COOH (oligomer) with *N*-Hydroxysuccinimide and the cholesterol derivative 2 (PNIPAM/NTHFAM-Chol or PNT-Chol)

2.3.4.6.1 Activated PNIPAM/NTHFAM-COOH

The same procedure was used, as described in section **2.3.4.5.1**, PNIPAM/NTHFAM-COOH (from section 2.3.3.4, 3.5 g, 1.84 mmol of COOH) was used together with *N*-hydroxysuccinimide (0.42 g, 3.6 mmol), DCC (1.56 g, 7.2 mmol) and DMAP (0.23 g, 1.84 mmol) in dry THF (80 mL). The solution was filtered twice to remove the precipitate. The oligomer was precipitated in diethyl ether (1 L). It was purified further by an addition precipitation from THF (~ 20 mL) into diethyl ether (1 L) and dried *in vacuo* (2.8 g, 80 %). IR (cm^{-1}, KBr): 3296, 3082, 2973, 2936.

2.3.4.6.2 Modification of the activated oligomer with the cholesterol derivative 2

The modification of activated PNIPAM/NTHFAM (0.5 g, 0.25 mmol) followed the procedure described in section **2.3.4.5.2**. The dry polymer was redissolved in water after precipitating three times from THF to ethyl ether. The solution was filtered through NALGENE filter (0.45 μm). The polymer was recovered by lyophilization of the solution (0.125 g, 25 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm) = 0.65 (s, 3H, cholesterol $3H_{18}$), 0.83-5.5 (m, cholesterol), 1.2 (s, 6H, NCHC$\underline{H}_3$), 1.30-3.90 (m, polymer backbone C$\underline{H}_2$ and protons from THFAM), 3.97 (broad s, 1H, NC$\underline{H}$), 6.7 (s, 1H, N$\underline{H}$). IR (cm^{-1}, KBr pellet): 3296, 3082, 2973, 2936, 2875, 1650, 1550, 1461, 1387, 1369, 1259, 1174, 1259, 1174, 1132, 1073, 1048, 926, 672.

2.3.5 Characterization of polymers

2.3.5.1 LCST determination by UV

The LCSTs of the polymers in aqueous solutions were determined by UV detection of the change in turbidity at 600 nm upon heating with a heating rate of 0.5 °C/min. The LCST with instrumentation error of ± 0.5 °C was taken as the temperature of the initial break point in the resulting transmittance versus temperature curve.

2.3.5.2 Molecular weight determination

Acid-base titration

Samples were prepared by dissolving the polymers (ca. 0.15 g) in 15 mL of $NaNO_3$ solution (0.01 M). 0.1 N NaOH aqueous solution was added to the samples to bring their pH to ca. 11. The samples were titrated with 0.1 N HCl at 25°C. A blank solution was titrated to determine the amount of 0.1 N HCl required to neutralizing the excess amount of NaOH.

Gel Permeation Chromatography (GPC)

The molecular weights and molecular weight distributions of PNIPAM and PNIPAM/THFAM were measured by GPC. A sample dissolved in dry THF (7 mg/mL) was eluted by THF at a flow rate of 1.0 mL/min. at 40 °C. Polystyrene standards were used for calibration. Molecular weights were thus estimated as those of polystyrenes of equivalent elution volume.

2.3.5.3 Dynamic light scattering for micelle size determination

Aqueous solutions (concentration range from 0.4-1.0 ± 0.05 g/L) of the polymers were prepared two days before the measurements. The water used to prepare the polymer solutions and the solutions were filtered with 0.2 and 0.45 μm filters, respectively to obtain dust free solutions.

2.3.5.4 Determination of the pyrene concentration by UV

The pyrene content of the labelled polymers was determined from UV absorbance of a solution of the polymer in methanol. The extinction coefficient of the pyrene units was assumed to be that of 1-Pyrenemethylamine (ε_{342}= 37,500, in methanol).

2.3.5.5 Estimation of the content of cholesterol incorporation on polymers

The content of cholesterol attached to PNIPAM and PNIPAM/NHFTAM chains was estimated from the H^1 NMR spectra by using the ratio of the area of the singlet at 0.65 ppm ($C\underline{H}_3$ from the cholesterol H_{18}) and the area of the singlet at 3.96-3.99 ppm (NH-C$\underline{H}$ from the polymer repeating units).

2.3.5.6 Fluorescence measurements

Solution preparation for labelled polymers

Stock solutions of the polymers were prepared one day before the measurements. Samples were made by dilution of the stock solutions of the polymers (ca. 0.5 g/L) to the

desired concentrations. The aqueous solutions were not degassed. Solutions in MeOH were degassed by argon for two-min. before the fluorescence measurements.

Preparation of pyrene-saturated water solution

A pyrene (purification procedure in section **2.1**) contained water suspension was stirred overnight in the dark at room temperature, then filtered. The value of I_1/I_3 of the pyrene-saturated water solution was 1.70-1.72 at excitation wavelength 334 nm.

Solution preparation for unlabelled polymers employing pyrene as probe

The preparation of aqueous solutions for most unlabelled oligomers and polymers was the same as for the labelled polymers, except that a pyrene-saturated water solution was used instead of water. Only one oligomer (PNIPAM-CHOL, PNIPAM end capped with cholesterol derivative 2 with Mn 2,000) sample was made with a special method due to its insolubility in water. This method was adopted from Sunamoto *et al.*[50] The oligomer (10-mg) was dissolved in DMSO (ca. 0.9 mL). Water (50 mL) was added slowly to the solution with constant stirring. The resulting solution was subjected ultrafiltration with a total 800 mL of water. The purified solution had an approximate volume of 7 mL and was used as a stock solution.

Fluorescence experiments

Fluorescence experiments were carried out with both the emission and excitation slit width set at 1.0 mm. Emission spectra were recorded with an excitation wavelength

of 344 nm for the Py-labelled polymers (both in aqueous solution and MeOH solution) and 334 nm for the unlabelled polymer using pyrene as probe with scans in the range of 360-600 and 360-550 nm, respectively. Excitation spectra were recorded for emission wavelengths of the monomer emission and excimer emission with scans in range of 260-390 nm.

Determination of the ratio of intensity of excimer emission and monomer emission (I_E/I_M)

The ratio of intensity of excimer emission and monomer emission (I_E/I_M) was calculated by taking the ratio of the maximum emission intensity at 474-480 nm to the half sum of the emission intensities I_1 (at ca. 376 nm) and I_5 (at ca. 396 nm).

Determination of critical aggregation concentrations (CAC)

The CACs of the labelled polymers in aqueous solutions were determined through emission spectra using fluorescence. The ratio of I_E/I_M was plotted as a function of polymer concentration (or cholesterol content incorporated on polymer chain). CAC was taken as the inflection point of the curves of the I_E/I_M *versus* polymer concentration.

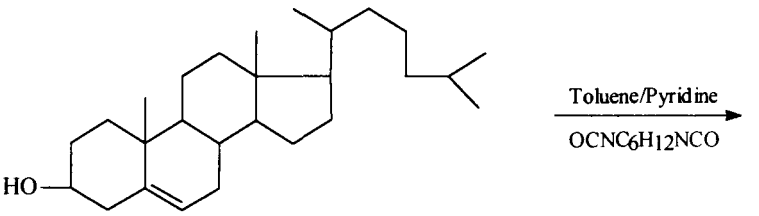
The CACs of unlabelled oligomers and polymers were determined by employing pyrene as probe. I_1/I_3 of the oligomer and polymer samples was plotted as a function of polymer concentration (or cholesterol content). The CAC was taken as the inflection point of the curve of I_1/I_3 *versus* polymer concentration.

3. Results and Discussion

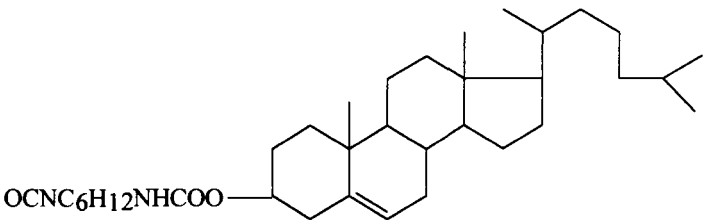
3.1 Syntheses and characterization of cholesterol derivatives

The attachment of functional groups to cholesterol was the first step towards the preparation of the cholesterol bearing oligomers and polymers. The functionalization of cholesterol included two steps: (1) the introduction of an isocyanate group to cholesterol through the 3β-hydroxyl group of cholesterol and (2) the introduction of an amino group by utilizing this new isocyanate group attached to the cholesterol molecule.

3.1.1 Cholesterol derivative 1 ($C_{35}H_{58}N_2O_3$, M=554)



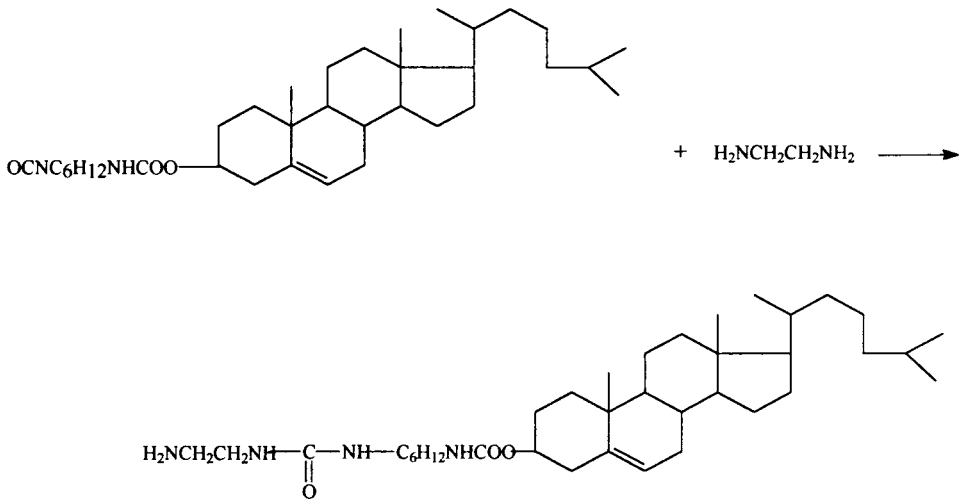
Cholesterol



Cholesterol derivative 1

A common approach to prepare a carbamate is by treatment of an isocyanate with an alcohol.[44] The procedure to prepare cholesterol derivative 1 was adapted from Sunamoto *et al.*[52] Cholesterol derivative 1 was synthesized through a condensation reaction of an isocyanate group (1,6-hexyldiisocyanate) and a hydroxyl group (3β-hydroxyl group from cholesterol). The molar ratio of 1,6-hexyldiisocyanate to cholesterol was 15:1 to ensure that only one isocyanate group from 1,6-hexyldiisocyanate reacted with the hydroxyl group of cholesterol. The yield of this reaction (63 %) was higher than the yield reported by Sunamoto *et al* (44 %).[52] The structure of the cholesterol derivative **1** was confirmed by the presence of a M+1 = 555 peak in its mass spectrum. The product was also characterized by IR and ^{1}H NMR spectroscopy. The IR spectrum showed a sharp band at 3345 cm^{-1} assigned to the N-H stretching, a strong band at 2254 cm^{-1} from the N=C=O stretching, a strong band at 1692 cm^{-1} from NHCOO stretching and a strong band at 1540 cm^{-1} from N-H bending. The information collected from the ^{1}H NMR spectrum also indicated the successful condensation reaction: a new signal (singlet) at 4.6 ppm (NH), a triplet at 3.27 ppm (CH_2NCO) and a doublet at 3.14 ppm (HN-CH_2).

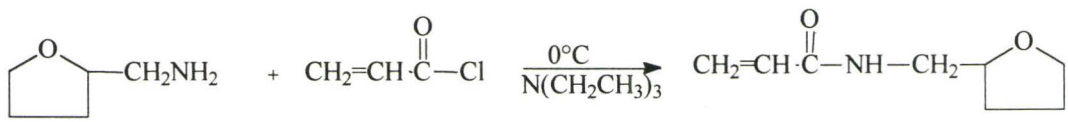
3.1.2 Cholesterol derivative 2 ($C_{37}H_{66}N_4O_3$, M=614)



Cholesterol derivative 2

Cholesterol derivative 2 was prepared through the condensation reaction of an isocyanate (cholesterol derivative 1) and a diamine (ethylene diamine). A large excess of ethylene diamine, relative to the cholesterol derivative 1, was used to ensure that only one amino group of the diamine molecule reacted with the isocyanate group in cholesterol derivative 1. The disappearance of the band at 2254 cm^{-1} in the IR spectrum confirmed that complete reaction took place between the isocyanate group and the amino group with the formation of a new product. In the IR spectrum a weak band at 3200 cm^{-1} together with a strong band at 3344 cm^{-1} confirmed the presence of a primary amine. A peak of M+1 =615 in the mass spectrum confirmed the structure of cholesterol derivative **2**. The ^1H NMR spectrum provided more evidence of the structure of the product: a 3-H singlet at 0.66 ppm (the protons from cholesterol H_{18}) and a 2-H peak at 1.23 ppm (the protons from the only one amino group left from diamine).

3.2 Synthesis and characterization of the monomer *N*-tetrahydrofurfurylacrylamide (NTHFAM)



***N*-Tetrahydofurfurylamine** **NTHFAM**

N-Tetrahydrofurfurylacrylamide (NTHFAM) was prepared by acylation of *N*-tetrahydrofurfurylamine with acryloyl chloride. This is a typical reaction of an acid chloride with an amine to form an amide, adapted from the Schotten-Baumann reaction.[44, 45] The molar ratio of amine to acryloyl chloride was 1:0.70. The excess *N*-tetrahydrofurfurylacrylamide was removed by two consecutive reduced pressure distillations. The inhibitor 2,6-di-*tert*-butyl-4-methyl-phenol was added to the reaction mixture to prevent free radical polymerization of the monomer NTHFAM that may be initiated by heating. The product obtained after the first reduced pressure distillation contained both monomer NTHFAM and the starting material *N*-tetrahydrofurfurylamine, as indicated in the ^{1}H NMR spectrum (a signal at 1.37 ppm, protons from $N\underline{H}_2$). In order to remove the small amount of residual *N*-Tetrahydrofurfurylamine, a second reduced pressure distillation was performed. NTHFAM is a monosubstituted alkene; in agreement with this structure the following splitting pattern was observed in the ^{1}H NMR spectrum in $CDCl_3$: the terminal vinylic hydrogens ($C\underline{H}_2$=CH) appeared at 5.6 (dd, J=10.3 Hz, J=1.5 Hz) and 6.55 (dd, J=17.0 Hz, J=10.3 Hz) ppm. The internal vinylic

hydrogen (CH_2=C<u>H</u>) was at 6.55 ppm (J=17.0 Hz, J= 1.5 Hz). The 1-H singlet at 7.34 ppm was attributed to CON<u>H</u>. It was confirmed by a ^{1}H NMR measurement in D_2O since the signal of CON<u>H</u> in $CDCl_3$ disappeared upon addition of D_2O due to deuterium exchange.

3.3 Synthesis and characterization of the polymers

All polymers in this work were synthesized through free radical polymerization. The hydrophobically modified polymers prepared here can be divided into two types based on their modification method. The first method is a copolymerization of NIPAM and NTHFAM with *N*-acryloxysuccinimide (NASI), a monomer containing a reactive group, followed by post modification of this copolymer with the cholesterol derivative 2. This method will give HM polymers with randomly distributed cholesterol groups on the polymer chain. The second method involves a polymerization in the presence of a chain transfer agent substituted by a functional group, followed by the post modification of this reactive polymer with the cholesterol derivative 2. The HM polymers made by this method have a cholesterol group at one end of the polymer chain.

Polymer abbreviations in this study are given in Table 3.1 and 3.2.

Table 3.1 HM-Polymers for study

Abbreviations	Meaning	Structure
PNIPAM-Chol	Cholesterol end-capped at one chain end of PNIPAM	Appendix. 1: 1.1
PNIPAM/NTHFAM-Chol (PNT-Chol)	Cholesterol end-capped at one chain end of copolymer NIPAM/NTHFAM (NIPAM/NTHFAM ratio:7/3)	Appendix. 1: 1.2
PNIPAM/Chol	Cholesterol randomly grafted on polymer chain	Appendix. 1: 1.3
PNIPAM/Py/Chol	Py labeled and cholesterol randomly grafted on polymer chain	Appendix. 1: 1.4
PNIPAM/NTHFAM/Chol (PNT/Chol)	Cholesterol randomly grafted on copolymer chain (NIPAM/NTHFAM ratio: 7/3)	Appendix. 1: 1.5
PNIPAM/NTHFAM/Py/Chol (PNT/Py/Chol)	Py labeled and cholesterol randomly grafted on copolymer chain (NIPAM/NTHFAM ratio: 7/3)	Appendix. 1: 1.6

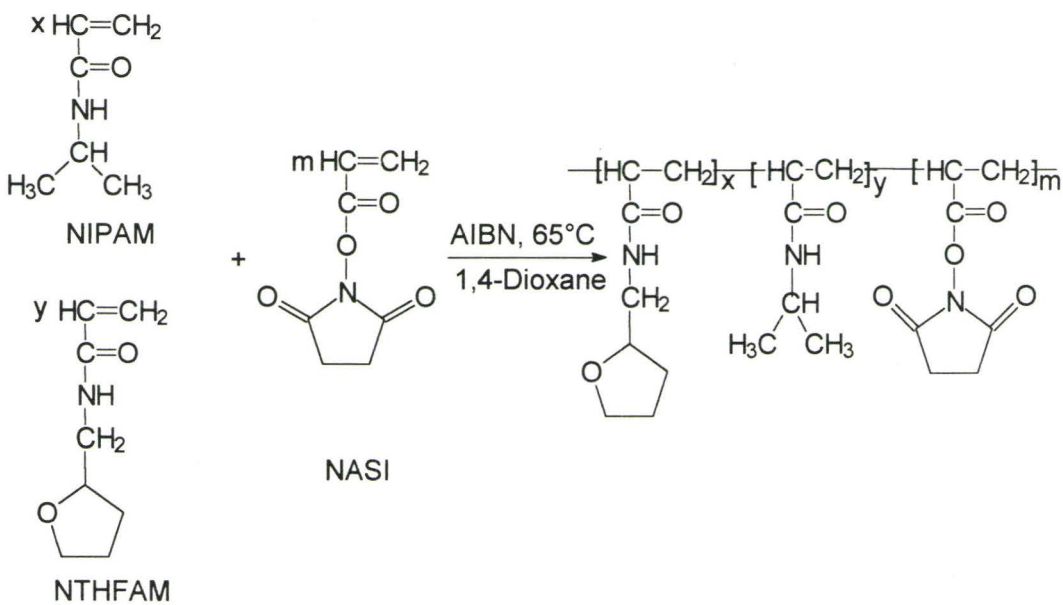
Table 3.2 Intermediates in this study

Abbreviations	Meaning	Structure
PNIPAM-COOH	COOH group end-capped at one chain end of PNIPAM	Appendix. 2: 2.1
PNIPAM/NTHFAM-COOH (PNT-COOH)	COOH group end-capped at one chain end of copolymer NIPAM/NTHFAM (NIPAM/NTHFAM ratio:7/3)	Appendix. 2: 2.2
PN_3T_7-COOH	COOH group end-capped at one chain end of copolymer NIPAM/NTHFAM (NIPAM/NTHFAM ratio:3/7)	Appendix. 2: 2.3
PNIPAM/NASI	Copolymer NIPAM/NASI (NIPAM/NASI ratio: 100: 2)	Appendix. 2: 2.4
PNIPAM/NTHFAM/NASI	Copolymer NIPAM/NTHFAM/NASI (NIPAM/NTHFAM/NASI ratio: 7/3/0.2)	Appendix. 2: 2.5
PNIPAM	Homo polymer	Appendix. 2: 2.6
PNTHFAM	Homo polymer	Appendix. 2: 2.7

3.3.1 Reactive copolymer PNIPAM/NASI and PNIPAM/NTHFAM/NASI

Both polymers were prepared according to the method of Hoffman[26] and F.M. Winnik [29] through a free radical polymerization (SCHEME 3.1). 1,4-Dioxane was chosen as solvent, based on its low chain-transfer rate and inertness toward NASI.[29,30] The molar ratio of monomer (NIPAM or both NIPAM and NTHFAM), NASI and initiator was 100/2/1 to obtain a polymer containing a small amount of reactive groups with a middle range of molecular weight.

Scheme 3.1 The synthetic route to the copolymer



For PNIPAM/NASI: X=100; Y=0; m=2
For PNIPAM/NTHFAM/NASI: X=70; Y=30; m=2

The signals due to succinimide group stretching were observed in the IR spectra of PNIPAM/NASI (at 1820, 1783, 1741, and 1716 cm^{-1}) and PNIPAM/NTHFAM/NASI (at 1808, 1783, 1741, and 1712 cm^{-1}). PNIPAM/NTHFAM/NASI also displayed a strong C-O stretching absorption at 1075 cm^{-1} resulting from the tetrahydrofurfuryl pendant group on polymer chains (Figure 3.1).

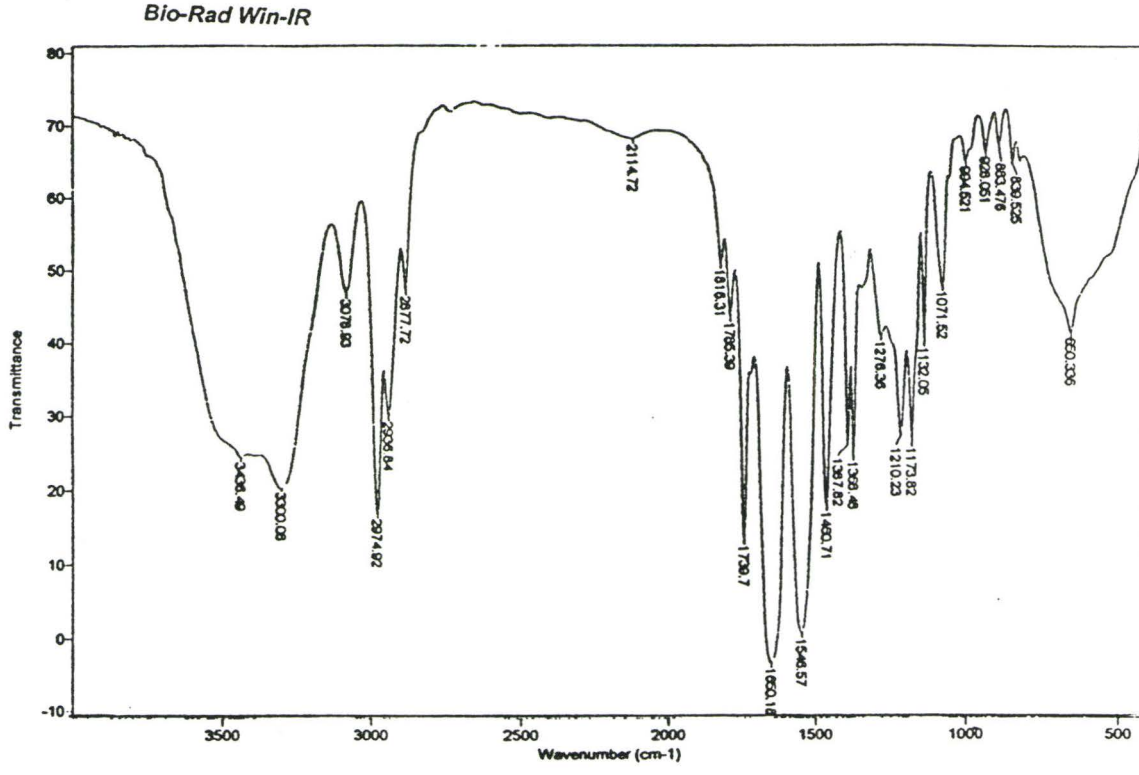


Figure 3.1 The IR spectrum of PNIPAM/NTHFAM/NASI

Molecular weight and molecular weight distribution of both reactive copolymers were measured by GPC. The results are shown in Table 3.3

Table 3.3 Molecular weights and molecular weight distributions of reactive copolymers*

Reactive Copolymer	Mn (Dalton) 10^{-4}	Mw(Dalton) 10^{-4}	Mw/Mn
PNIPAM/NASI	1.7	4.4	2.6
PNIPAM/NTHFAM/NASI	0.4	2.1	5.3

* By GPC using polystyrene standards.

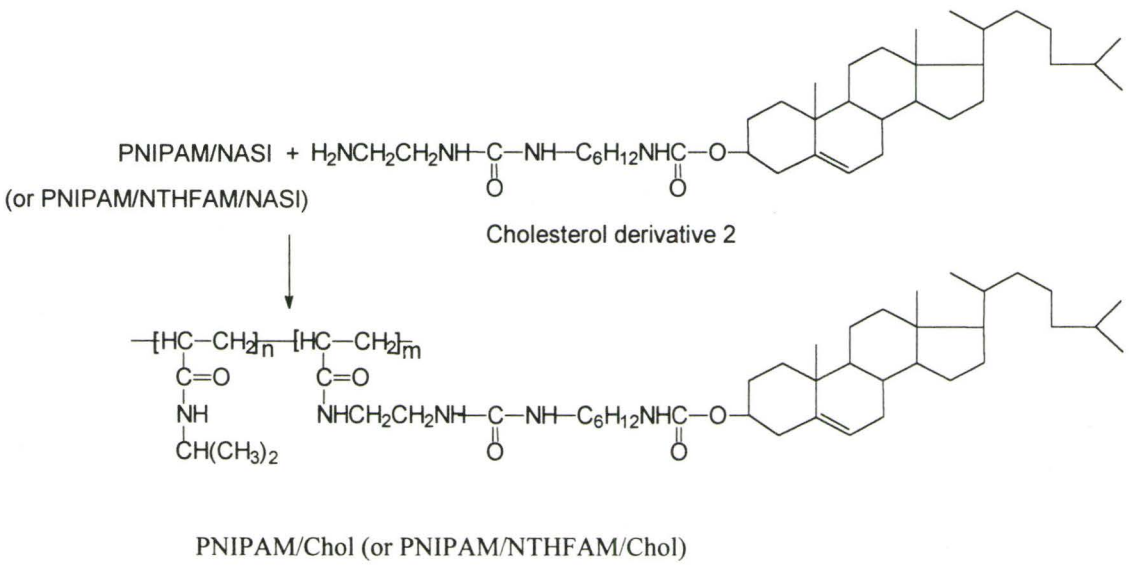
The molecular weight distributions of both copolymers are broad due to the nature of free radical polymerization. Under similar reaction condition and same ratio of monomer and initiator, the molecular weight of PNIPAM/NTHFAM/NASI obtained was lower with wider molecular weight distribution than that of PNIPAM/NASI. The conversion was about 70 % for PNIPAM/NASI and 85 % for PNIPAM/NTHFAM/NASI. As a rule, the higher conversion rate of the same monomer under the same conditions (molar ratio of monomer and initiator, temperature, etc.), the wider the molecular weight distribution of polymer obtained by free radical polymerization.[5, 20] Although NHTFAM belongs to the family of *N*-substituted acrylamide, co-polymerization of NIPAM, NTHFAM and NASI has not been reported previously. More experiments are needed to assess the relationship between the molecular weight and its distribution, the conversion rate and the molar ratio of monomer and initiator.

3.3.2 Modification of PNIPAM/NASI and PNIPAM/NTHFAM/NASI

3.3.2.1 Modification of copolymers with cholesterol derivative 2

The modification of copolymers was done by reaction of the cholesterol derivative 2 with the copolymers PNIPAM/NASI and PNIPAM/NTHFAM/NASI, respectively (Scheme 3.2).

Scheme 3.2 The post modification of PNIPAM/NASI and PNIPAM/NTHFAM/NASI



The ratio of monomer to cholesterol derivative 2 attached to the polymer was estimated by analysis of ^{1}H NMR spectrum. Figure 3.2 shows the ^{1}H NMR spectrum of PNIPAM/Chol. The singlet at ca. 0.65 ppm is due to the three hydrogen atoms of the methyl group in cholesterol (H_{18}). The proton from the monomer NH-C<u>H</u>$(CH_3)_2$ also gives a well resolved singlet at ca. 3.96 ppm. The average numbers of incorporation of cholesterol derivative 2 on the polymer chain were obtained by taking the ratio of

monomer repeating units to cholesterol derivative 2 group by comparing the area under these two peaks (Table 3.4).

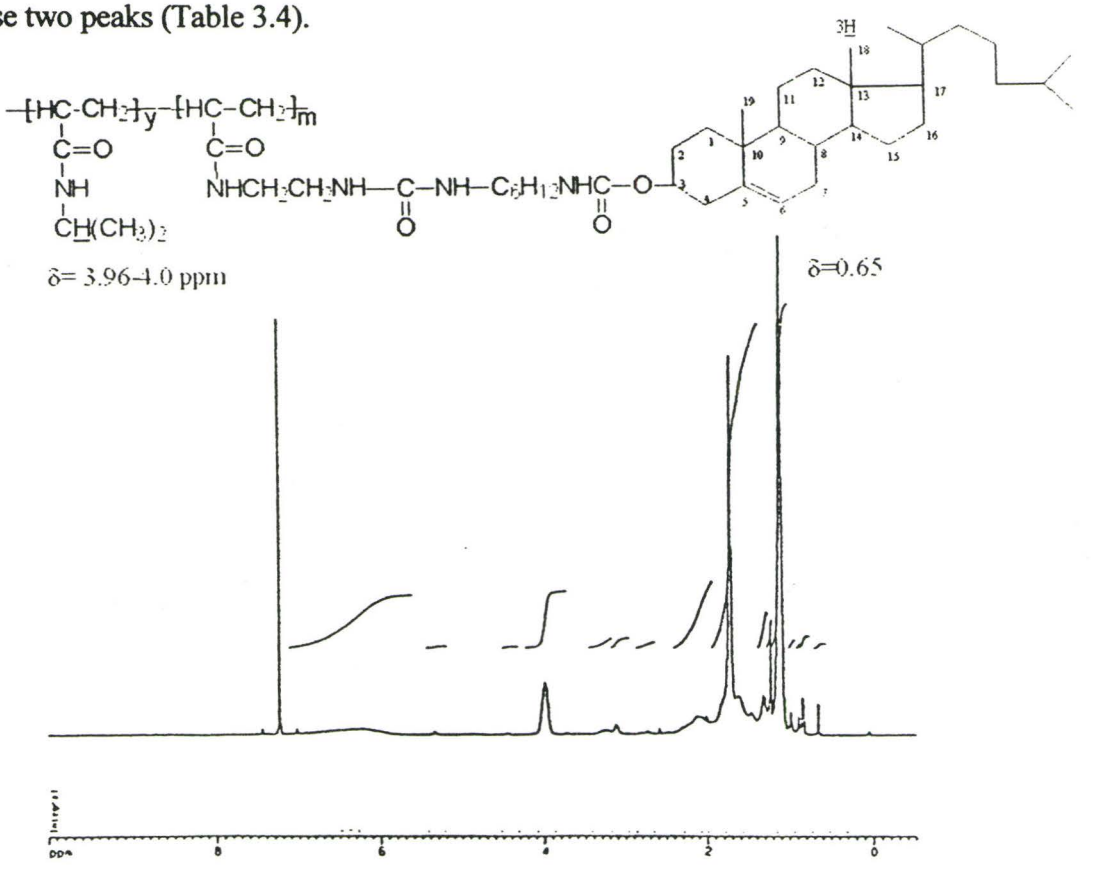


Figure 3.2 ^{1}H NMR spectrum of PNIPAM/Chol in $CDCl_3$

Table 3.4 The Estimation of incorporation of cholesterol derivative 2 in copolymer chain

Copolymer	The molar ratio of monomer units to cholesterol derivative 2 group*
PNIPAM/NASI/Chol	57:1
PNIPAM/THFAM/NASI/Chol	50:1

* by ^{1}H NMR spectrum in $CDCl_3$.

3.3.2.2 Modification of copolymers with cholesterol derivative 2 and pyrene derivative

This modification was performed using the procedure discussed in **3.3.2.1** (Scheme 3.2), starting with the same copolymers (PNIPAM/NASI and PNIPAM/NTHFAM/NASI) which were modified with two hydrophobic groups: the cholesterol derivative 2 and 1-pyrenemethyl amine hydrochloride. The copolymers were reacted first with the pyrene derivative and then cholesterol derivative 2. The estimated molar ratio of monomer and incorporated cholesterol derivative 2 (Table 3.5) was obtained by the ^{1}H NMR method described in the previous section. The pyrene content on the polymer chain was calculated from the UV absorption of the pyrene group (see Table 3.3).

Table 3. 5 The estimation of chemical composition of copolymers

Copolymer	Composition (unit mol) Monomer: Py: Chol	[Py] (mol/g) x 10^5	[Chol] (mol/g) x 10^4
PNIPAM/Py/Chol	162: 1: 2.2	5.5 ± 0.1	1.2 ± 0.1
PNIPAM/THFAM/Py/Chol	179:1: 2.3	4.5 ± 0.1	1.0 ± 0.1

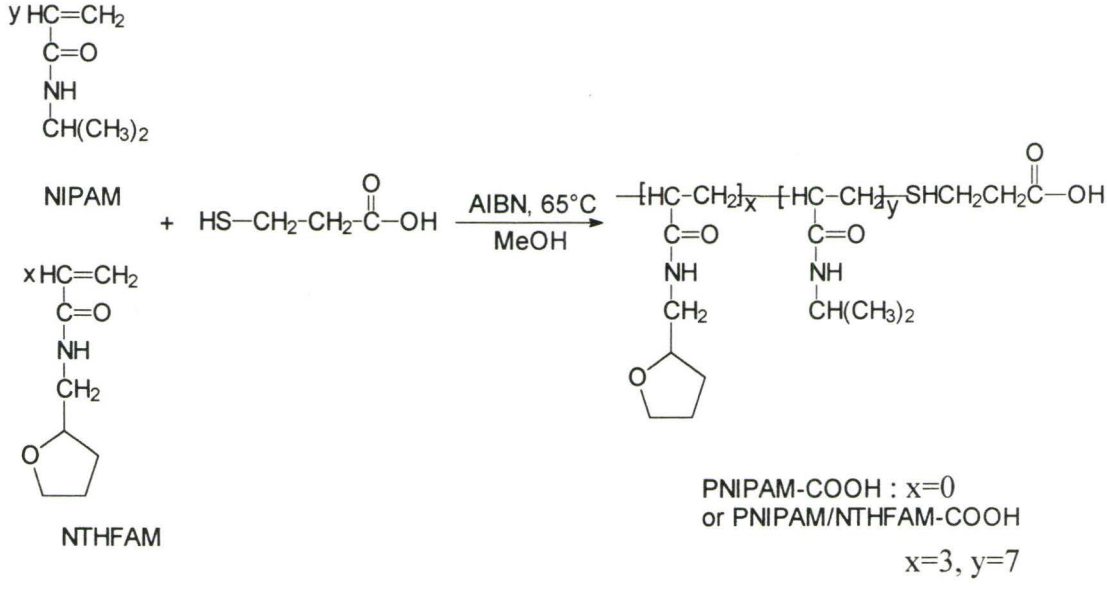
[a] by ^{1}H NMR spectra in $CDCl_3$; [b] by UV spectra

3.4. Synthesis and characterization of oligomers

The preparation of the end-functionalized oligomers PNIPAM and PNIPAM/NTHFAM (co-oligomer) consisted of three reactions: (1) free radical polymerization in the presence of a chain transfer agent to prepare a carboxyl-terminated oligomer; (2) activation of the carboxyl-terminated oligomer by N-hydroxysuccinimide; and (3) coupling reaction of the cholesterol derivative 2 to the oligomer chain.

PNIPAM-COOH and PNIPAM/NTHFAM-COOH were obtained by free radical polymerization (Scheme 3.3), as described by A. Yamazaki.[35]

Scheme 3.3 Synthetic route to the carboxyl-ended PNIPAM or PNIPAM/NTHFAM



Mercaptopropionic acid was used as the chain transfer agent to control the molecular weight and to attach a carboxyl group at the chain end. The reaction

conditions and the molar ratio of monomer, initiator and chain transfer agent play key roles in controlling the molecular weight of the oligomers. Table 3.6 gives the molecular weights of the oligomers, which were made with different molar ratio of monomer, initiator and chain transfer agent but similar reaction temperature and time. The molecular weights of oligomers were determined by acid/base titration of the terminal carboxyl group.

Table 3.6 Oligomer characterization

Oligomer	Molar ratio [M]:[I]:[CT][a]	Mn[b] (average)
PNIPAM-COOH	100:1:8	1,950 ±100
	100:1:2.2	8,500±100
PNIPAM/NTHFAM-COOH (NIPAM/NTHFAM:7/3)	100:1:8	1950±100

[a] [M]:[I]:[CT] = [Monomer]:[Initiator]:[Chain transfer agent]

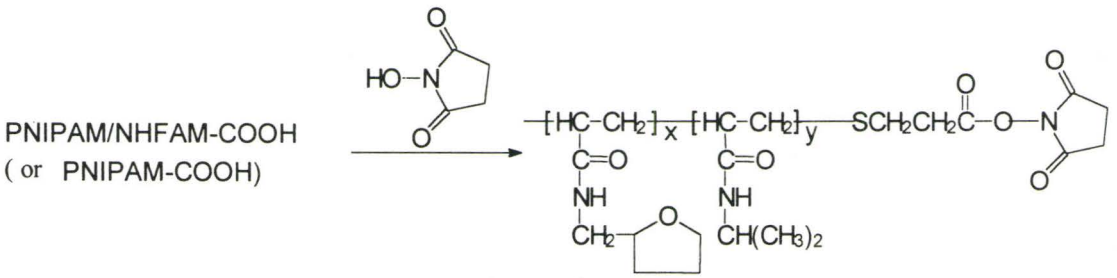
[b] by acid/base titration.

With a molar ratio of monomer, initiator and chain transfer agent (100:1:8) under similar reaction condition, the molecular weight of the oligomers is close to 2,000 and similar to that reported by A. Yamazaki[35] with similar conditions. PNIPAM-COOH with higher molecular weight (8,500) was prepared by keeping the same molar ratio of monomer and initiator, but reducing the amount of chain transfer agent. A similar ratio

used by A. Yamazaki[35] (M: I: CT=100:1:2, yield 27 %) yielded polymer with molecular weight ca. 14,600, which was much higher. This might be due to the much higher conversion rate (75 %) achieved in our case.

In order to couple the cholesterol derivative 2 to the end of polymer chain, oligomers with carboxyl end group have to be activated.[35, 44, 49] This activation was accomplished by reacting PNIPAM-COOH and PNIPAM/NTHFAM-COOH with *N*-hydroxysuccinimide (Scheme 3.4). IR spectra of both activated PNIPAM and PNIPAM/NTHFAM showed bands at 1816, 1785 1739 cm^{-1}, due to CO-N-CO stretching vibration of the *N*-Succinimide group.

Scheme 3.4 The activation of PNIPAM-COOH and PNIPAM/NTHFAM-COOH

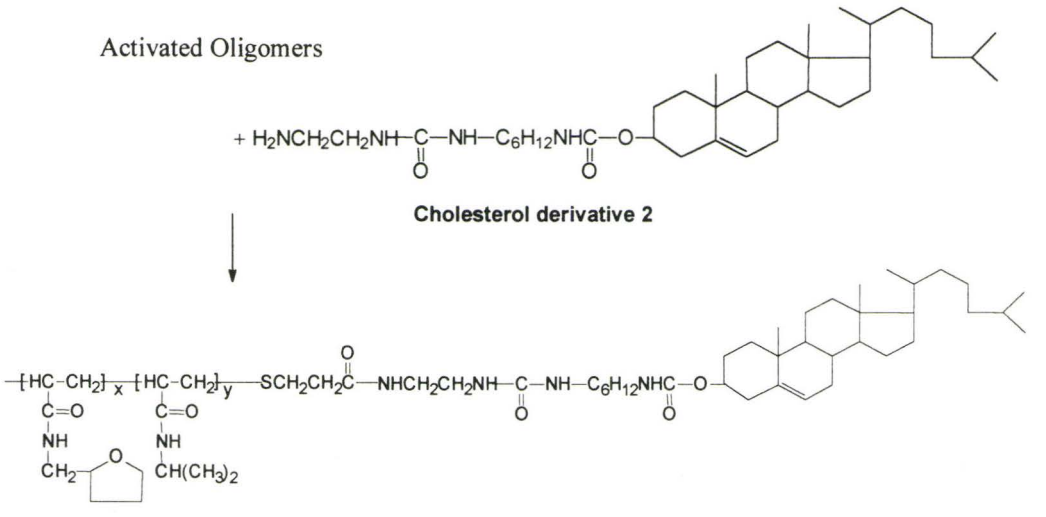


The activated oligomers

The coupling reaction of the activated oligomers with the cholesterol derivative 2 (Scheme 3.5) was conducted for 24 h and monitored by TLC. The unreacted activating group was quenched by isopropylamine at the end of the reaction. The cholesterol coupling efficiency was estimated from the ^1H NMR spectra of PNIPAM-Chol and PNIPAM/NTHFAM-Chol. Table 2.5 shows the monomer repeating units calculated by

both acid/base titration method and ^{1}H NMR results based on the molar ratio of monomer units and cholesterol group. The acid/base titration method gives the number of monomer-repeating units; 18 for PNIPAM-COOH and 16 for PNIPAM/NTHFAM-COOH, respectively. The calculation of molar ratio of monomers and cholesterol gives a larger number of monomer repeating units, which indicates that the coupling reaction was not 100 %. The coupling efficiency is defined as the fraction of carboxylated polymers that was converted to cholesterol bearing polymers. From the results listed in Table 3.7, the coupling efficiency is calculated to be ca. 47.4 % for PNIPAM-Chol and 69.6 % for PNIPAM/NTHFAM-Chol, respectively.

Scheme 3.5 The coupling of activated oligomers with cholesterol derivative 2



PNIPAM-Chol (or PNIPAM/NTHFAM-Chol)

Table 3.7 The estimation of end coupling efficiency of PNIPAM-Chol and PNIPAM/NTHFAM-Chol

Oligomer	Mn[a] (repeating units)	The molar ratio of monomer units to cholesterol derivative 2 group by ^{1}H NMR	Estimation of coupling efficiency, %
PNIPAM-CHOL	2,000 (ca. 18)	38:1	47.4
PNIPAM/NTHFAM-CHOL (NIPAM/NTHFAM:7/3)	1950(ca. 16)	23:1	69.6

[a] by acid/base titration.

3.5 The solution properties of polymers and oligomers

As shown in Table 3.8, the intermediates, as well as PNIPAM with a molecular weight ca. 44,000 containing less than 2 % (in molar ratio) NASI and others were characterized together with the polymers of interest here, for comparison purpose. The LCST measured for PNIPAM was ca. 32.5 ° C, similar to the reported value.[17, 27]

Table 3.8 The physical properties of oligomers and polymers

Oligomers and polymers (Structures in Appendix. 1 & 2)	Type of oligomers and polymers	Mn[a] (Dalton)	LCST (°C)[b]
PNIPAM	Containing less than 2% of NASI	Mw[e]: 44,000	32.5
PNIPAM-COOH	End-capped with COOH	2,000 8,500	34.5 33.0
PNT-COOH (PNIPAM/NTHFAM-COOH) (Monomer ratio: 7/3)	End-capped with COOH	2,000	36.0
PN_7T_3-COOH (Monomer ratio: 3/7)	End-capped with COOH	1,800	38.5
PNTHFAM-COOH	End-capped with COOH	1,600 3,100	60.0 60.0
PNIPAM/NTHFAM (Monomer ratio: 7/3)	Copolymer of NIPAM/NTHFAM containing less than 2% of NASI	Mw[e]: 21,000	36
PNTHFAM[42]	Homopolymer	Mv[f]: 31,000	63

[a] by acid/base titration; [b] by UV measurement; [c] by ^{1}H NMR spectra.
[e] by GPC [f] by Ubbelohde viscometer

Two factors may be responsible for the increase of the LCST in the case of carboxylic terminated oligomer NIPAM, compared to PNIPAM: (1) relatively low molecular weight and (2) the carboxylic end group. Schild and Tirrell observed that the LCST of PNIPAM decreases with increasing of molecular weight.[19] PNIPAMs terminated with carboxylic group (PNIPAM-COOH) with different molecular weight were prepared (Table 3.8). The lower Mn polymer has a higher LCST, reflecting the two effects. The end hydrophilic group affects the balance of hydrophilic and hydrophobic forces in a short oligomer, however, this effect is weakened in longer molecules. Thus PNIPAM-COOH with Mn ~8,500 showed LCST at 33°C, which was very closed to value of PNIPAM with high molecular weight (32.5°C).

The NTHFAM monomer unit is more hydrophilic than NIPAM due to the presence of an ether oxygen. The oligomer NTHFAM chains can form hydrogen bonds with water molecules at two types of hydrophilic sites (the amide and the alkoxy group). As a consequence, PNTHFAM-COOH and PNTHFAM showed a much higher LCST, ca. 60 - 63 °C (listed in Table 3.8). Co-polymerization of NIPAM and NTHFAM not only introduced the hydrophobic group (isopropyl from NIPAM), but also reduced the amount of alkoxy group (the strong hydrophilic site from NTHFAM). Two feed ratios of NIPAM and NTHFAM (3/7 and 7/3) were used to produce co-polymers and co-oligomers with different compositions. The exact determination of the compositions of the final co-polymers and co-oligomers by ^{1}H NMR could not be achieved, due to the overlap of proton signals from both monomers. The LCSTs of the copolymers and co-oligomers with 3/7 (PN_3T_7-COOH) and 7/3 (PNT-COOH and PNIPAM/NHTFAM) feed

ratios are ca. 38.5 °C and 36 °C, respectively. The results show that the LCST of NIPAM/NTHFAM copolymer is related to their composition: a higher NIPAM feed ratio results in a LCST value for the copolymer close to that of homogenous PNIPAM (ca. 32.5°C). In this PNIPAM/NTHFAM system, it should be noticed that the LCST of the copolymer of PNIPAM/NTHFAM (3/7) was much lower (ca. 39 °C) than that of homogenous PNTHFAM (ca. 60 °C), while the LCST of copolymer of PNIPAM/NTHFAM (7/3) was ca. 36 °C, not too much change from 39 °C, a value very similar to that observed for the solution of the copolymer with a significantly lower amount of NTHFAM. Because of limitation and the objectives of this study, the understanding of this issue was left at this level.

The functionality, molecular weight and LCST of HM-oligomers and HM-polymers are listed in Table 3.9.

Table 3.9 The physical properties of HM-oligomers and HM-polymers

Polymers	Type of polymer (Structure in Appendix. 1 & 2)	Mw [a]	LCST (°C)[c]	[Py][d] (mol/g)	[Chol][e] (mol/g)
PNIPAM-Chol	Cholesterol end capped on oligomer chain	Mn[b] 2,000	<33	No	2.4×10^{-4}
PNIPAM/NTHFAM-Chol	Cholesterol end capped on oligomer chain	Mn 1,950	35.5	No	3.6×10^{-4}
PNIPAM/Chol	Cholesterol derivative grafted randomly	44,000	31	No	1.5×10^{-4}
PNIPAM/Py /Chol	Cholesterol derivative and pyrene grafted randomly	44,000	32	5.5×10^{-5}	1.2×10^{-4}
PNIPAM/NTHFAM /Chol Monomer ratio: 7/3	Cholesterol derivative grafted randomly	21,000	~34	No	1.6×10^{-4}
PNIPAM/NTHFAM /Py/Chol Monomer ratio: 7/3	Cholesterol derivative and pyrene grafted randomly	21,000	~35	4.5×10^{-5}	1.0×10^{-4}

[a] by GPC; [b] by acid/base titration; [c, d] by UV measurements; [e] by ^{1}H NMR spectra.

In the case of HM PNIPAM, both hydrogen bonding and hydrophobic interactions affected the LCST.[27] As a rule, the LCST of a polymer in water decreases with increasing hydrophobicity.[27, 76] All the cholesterol modified PNIPAMs and the PNIPAM oligomers, however, showed only a slightly depressed LCST, compared with the unmodified analogs. This is quite similar to the result reported by F.M. Winnik et al,[30] that PNIPAM modified with C_{18} alkyl group and pyrene group (206: 1: 1) showed a LCST very close to that of PNIPAM. The HM polymers form micelles with a hydrophobic core and hydrophilic shell. Accordingly, it expected that the new attached hydrophobic groups might form the core of the micelle and are insulated from water and therefore made little contribution to the LCST.[30] Attaching cholesterol to the copolymers and cooligomers of NIPAM and NTHFAM resulted in nearly no change on the LCST. There were two factors to be considered in the NIPAM/NTHFAM (7/3) system: either (1) the above formation of micelles and /or (2) the balance of hydrophilic site (amide and alkoxy group) and the hydrophobic site (isopropyl group) on the polymer chain.

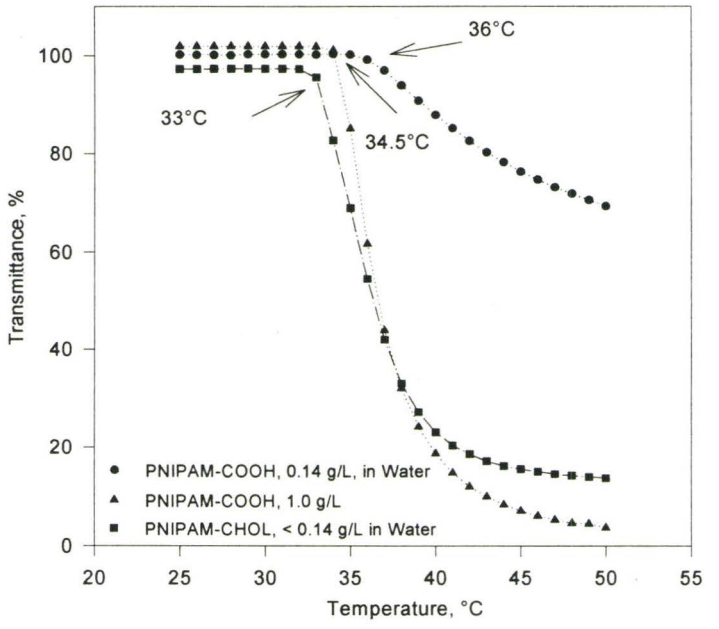


Figure 3.3 The LCSTs of PNIPAM-COOH and PNIPAM-Chol

All HM oligomers and HM polymers are soluble in water up to concentrations of 10 g/L, except PNIPAM-Chol which is almost insoluble in water. Figure 3.3 shows the curves of UV transmittance of aqueous solutions of PNIPAM-COOH with two concentrations and its cholesterol-modified oligomer against temperature. The LCST is taken at the breaking point at which the UV transmittance (ca. 600 nm) decreases. There are two curves of PNIPAM-COOH obtained with solutions of different polymer concentrations. The value of LCST is 34.5 °C for the solution of concentration 1.0 g/L (the curve with solid triangles) but 36° C for the solution of concentration 0.1 g/L (the curve with solid dots) with a broad curve that indicates that the change of turbidity at

such low concentration is not large enough for detection due to the instrumental sensitivity by UV. Usually the concentration of polymer aqueous solutions for LCST determinations by UV should be about 0.5-1.0 g/L in order to give a reliable result. The low concentration of PNIPAM-COOH used in this case for LCST measurement was chosen to allow the comparison of this sample with its cholesterol modified derivative, since the latter is insoluble at higher concentration. The third curve (with solid squares) in Figure 3.3 is PNIPAM-Chol. The aqueous solution of PNIPAM-Chol was diluted to 0.14 g/L and still not well dissolved. The undissolved portion was separated by filtration, and the filtrate was used for cloud point determination. The cholesterol-modified oligomer (PNIPAM-CHOL) with very low concentration (< 0.14 g/L) had an LCST ca. 33°C with a sharp decreasing in UV transmittance. It is clear that the LCST is perturbed by the hydrophobic interaction between the end capped cholesterol derivative on the oligomer chains and water, even at such a low concentration.

Dynamic Light Scattering (DLS) measurements provided strong evidence of the formation of aggregates or micelles in aqueous solution of HM-polymers and HM-oligomers (Table 3.10 and Table 3.11). Previous report from Winnik *et al* [33,37] established that PNIPAM aqueous solution (5 g/L) give no signal in DLS measurement at a fixed angle. By contrast aqueous solutions of all our cholesterol bearing polymers below LCST show strong signals in the similar DLS measurements, indicating the formation of micellar structures. The micelle sizes are reported as mean diameters. The polydispersity values of the micellar sizes show a broad distribution of the micelle sizes. Usually the randomly HM polymers have smaller micelles (smaller mean diameter) than

the end-HM polymers (larger mean diameter), as reported by Winnik *et al* [33, 37] in their work with C_{18} bearing PNIPAMs. In our case, we observed the similar phenomenon (Table 3.10 for cholesterol randomly grafted on polymer chain and Table 3.11 for cholesterol end capped on polymer chain). As shown in Table 3.9, the concentration of PNIPAM-Chol used for the DSL measurement is ca. 0.7 g/L, a solution of such high concentration had to be prepared by a special method[52] (described in **Experimental Section: 2.3.5.6**). So it may be worth to do further DSL measurements on this sample with a series of diluted concentrations, in order to compare the DSL results with other cholesterol bearing polymers.

Table 3.10 DLS results of randomly HM-polymers

HM-polymers	Mean diameter (nm)	Polydispersity
PNIPAM/Chol (0.8 g/L)	92±10	0.25±0.02
PNIPAM/Py/Chol (0.8 g/L)	65±10	0.28±0.02
PNIPAM/NTHFAM/Chol (1.0 g/L)	45±10	0.17±0.03
PNIPAM/NTHFAM/Py/Chol (1.0 g/L)	24±5	0.18±0.03

Table 3.11 DLS results of end-capped HM-oligomers

HM-oligomers	Mean diameter (nm)	Polydispersity
PNIPAM-CHOL (0.7 g/L)	175±10	0.20±0.03
PNIPAM/NTHFAM-CHOL (0.7 g/L)	158±10	0.23±0.05

3.6 Solution properties of HM-oligomers and HM-polymers:

A study via fluorescence methods

All polymers and oligomers were studied by fluorescence to collect further evidence of the formation of micellar aggregates in their aqueous solution. In the case of pyrene-labeled polymer, the dye attached to the polymer was used in the measurements. For the polymers without intrinsic pyrene group, a probe, pyrene was added to the polymer aqueous solutions for fluorescence study.

3.6.1 Unlabeled cholesterol bearing oligomers and polymers

Four unlabeled cholesterol bearing oligomers and polymers were studied with pyrene probe. As discussed in the introduction section, pyrene has a very low solubility in water ($\leq 10^{-7}$ M). In the presence of micelles, it is preferentially solubilized in or near the interior hydrophobic region of the micelles. The value of I_1/I_3 was used to investigate the formation of micelles in HM oligomers and polymers aqueous solution.

Oligomer PNIPAM-Chol (Mn 2,000) and PNIPAM/NTHFAM-Chol (Mn 1,950)

The variations of I_1/I_3 in aqueous solutions of two hydrophobically modified oligomers and their unmodified analogs are plotted as a function of oligomer concentration (Figure 3.4).

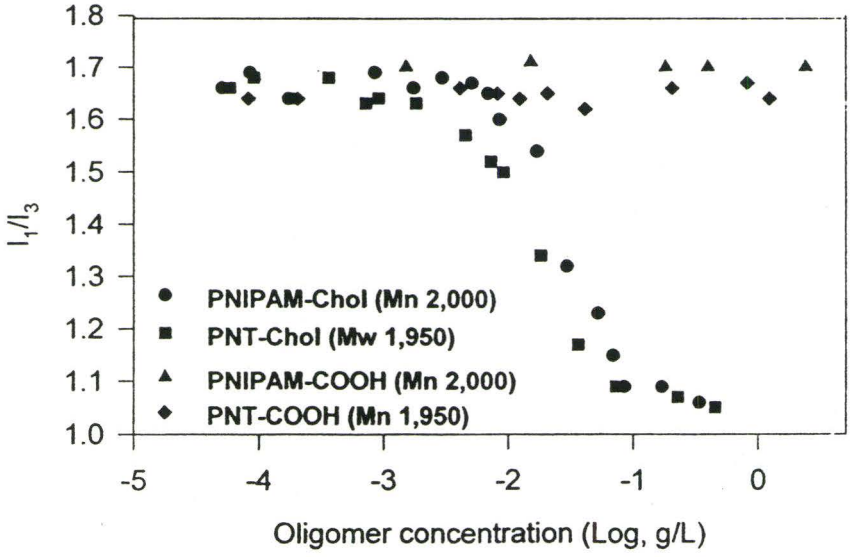


Figure 3.4 I_1/I_3 of oligomers as a function of oligomer concentrations

The I_1/I_3 of both carboxyl-terminated oligomers PNIPAM-COOH and PNIPAM/NTHFAM-COOH was constant, close to that of pyrene in water, over all the concentration range. This indicates that the probe is in a non-micellar environment and confirms the absence of polymer association in the solutions of these polymers.

For aqueous solutions of the HM oligomers, a polt of I_1/I_3 as a function of oligomer concentration consisted of three regions: (I) at very low concentration (polymer concentration < ca. 0.0032 g/L, cholesterol content <1.2-1.5 × 10^{-6} mol/L), I_1/I_3 takes a value close to that of pyrene in water; (II) in the intermediate concentration (polymer concentration ca. 0.0032 g/L cholesterol content 1.2-1.5 × 10^{-6} mol/L), it decreases

rapidly with increasing oligomer concentration (cholesterol content); and (III) at high concentration (polymer concentration 0.04-0.05 g/L, cholesterol content ca. 2-2.3 $\times$ 10^{-5} mol/L), it reaches a plateau (ca. 1.05). In the first region, Py probe stays in aqueous solution and senses a non-micellar environment. In the second region, the molecules of HM oligomers start to associate and the micellar structure is forming. The solubilization of pyrene into a hydrophobic environment is detected by a decrease in I_1/I_3. In the third region, the association of polymer is saturated and the micelles which exist in equilibrium with oligomer become the predominating species

The critical aggregation concentrations (CACs) of HM oligomers were determined by taking the first breaking point (in the vicinity of the region I and II) of I_1/I_3 where the ratios started to decrease with increasing oligomer concentration. This breaking point corresponds to the critical concentration where the intermolecular aggregation of cholesterol starts to occur. There can not be any intramolecular interaction between the cholesterol group on the same chain in these HM oligomers since they are end-capped with a cholesterol group at one end of chain. At the CAC, the pyrene probe is incorporated into the hydrophobic microdomains (the end-capped cholesterol group). In this environment it is protected from quenchers dissolved in water (e.g. Oxygen) and consequently its overall emission increases. The CAC values are 1.2 $\pm$ 0.5 $\times$ 10^{-6} mol/L (cholesterol content) for PNIPAM-Chol and 1.5 $\pm$ 0.5 $\times$ 10^{-6} mol/L (cholesterol content) for PNIPAM/NTHFAM-Chol. The CACs of PNIPAM-Chol and PNIPAN/NTHFAM-Chol are almost same, which suggests that the cholesterol content is a dominant fact in the polymer association.

PNIPAM/Chol (Mw 4.4 x 10^4) and PNIPAM/NTHFAM/Chol (Mw 2.2 x 10^4)

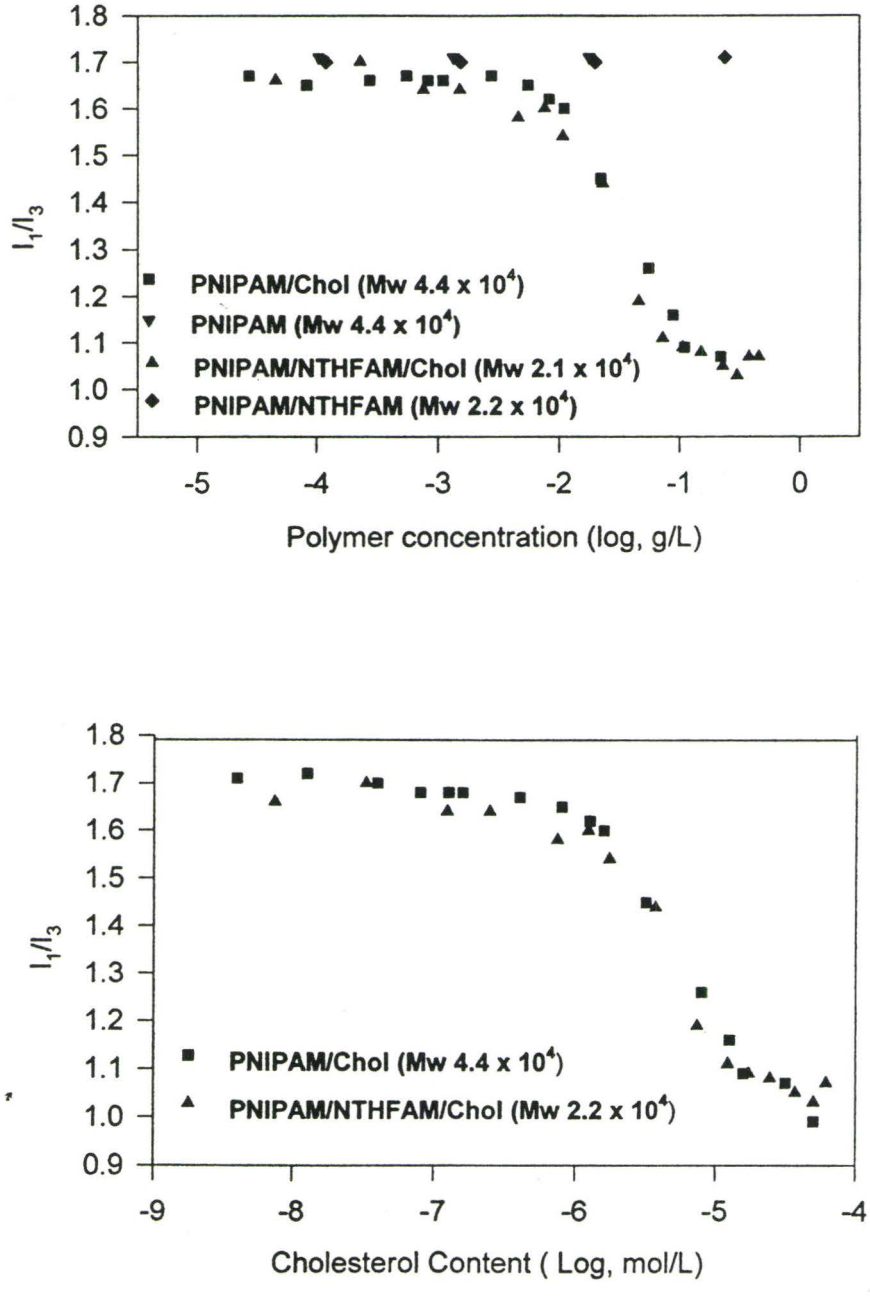


Figure 3.5 I_1/I_3 of polymers as a function of polymer concentrations (top) and cholesterol content(bottom)

Figure 3.5 shows the changes of I_1/I_3 as a function of polymer concentration for PNIPAM/Chol, PNIPAM/NTHFAM/Chol and their unmodified analogs. The following trends observed: The ratios I_1/I_3 of pyrene in solutions of the unmodified polymers remain constant with a value similar to that of pyrene in water (ca. 1.70) over the entire polymer concentration range probed and implies that pyrene resides in water and is not solubilized in a micellar environment. It is confirmed that the unmodified polymers do not form micelles in aqueous solution, as already observed in the case of the unmodified oligomers (in Figure 3.4). The variations of I_1/I_3 vs. concentrations of the HM polymers exhibit three regions, similar to those observed in the case of hydrophobically modified oligomers. The ratios I_1/I_3 of pyrene in solutions of HM polymers at very low concentration (region I) is constant (ca.1.65) but slightly lower than that of pyrene in water (ca. 1.70). The value of I_1/I_3 of HM polymers decreases abruptly as the concentration of HM polymer increases (region II) then reaches a plateau ca. 1.06 (region III).

The first breaking point (between region II and I) of I_1/I_3 of HM polymers corresponds to a cholesterol content ca. $1.3 \pm 0.5 \times 10^{-6}$ mol/L (polymer concentration: 0.0083 ± 0.00005 g/L) for PNIPAM/Chol and ca. $1.7 \pm 0.5 \times 10^{-6}$ mol/L (polymer concentration: 0.0076 ± 0.00005 g/L) for PNIPAM/NTHFAM/Chol. These values are taken as the CACs of HM polymers. The CACs are considered as the lowest polymer concentration for which the cholesterol groups start associating each other and forming micellar-structure. Apparently, at cholesterol content (ca. 1.3×10^{-6} mol/L) lower than CAC, only intramolecular aggregates exist, which may account for the slightly lower I_1/I_3

(1.65) detected. Beyond the CAC both intra- and intermolecular interactions exist in HM polymer aqueous solutions. Although the two polymers have different chemical composition, their CACs in terms of cholesterol content are in the same range and also similar to that of observed for the HM oligomers and this suggests that cholesterol content is the dominant in the formation of polymeric micelles.

Comparison of the results of HM oligomers and HM polymers

Two HM oligomers and two HM polymers have been studied employing pyrene as probe and their CACs were determined through this technique. It was extremely interesting to observe that the cholesterol terminated PNIPAM oligomers, the random copolymer of NIPAM and NTHFAM (7/3), and the cholesterol randomly attached PNIPAM, PNIPAM/NHTAM all have CAC in the similar range (1.3-1.7 $\times$ 10^{-6} mol/L) (Figure 3.6). The results suggests that it is the nature of cholesterol group who plays a leading role in the onset of cholesterol aggregation occurring at 1.3-1.7 $\times$ 10^{-6} mol/L (cholesterol content). The aqueous solution of those HM oligomers and polymers start forming micellar structures between polymer chains through the hydrophobic interaction at cholesterol content 1.3-1.7 $\times$ 10^{-6} mol/L. F.M. Winnik *et al* reported a similar phenomenon on the behavior of end alkylated PNIPAM and randomly alkylated PNIPAM.[30-34] They observed that the micropolarity sensed by pyrene is the same in solutions of the end-alkylated copolymers and randomly alkylated copolymers over the entire concentration range, with the onset of micelle formation occurring at octadecyl group concentration ca. 4 $\times$ 10^{-5} mol/L. It was concluded that the hydrophobic forces

(octadecyl chains) were the predominant role in the micellization process. The CAC of C_{18} bearing PNIPAM is ten times higher than that of cholesterol modified PNIPAM since cholesterol group is more hydrophobic than C_{18} group.

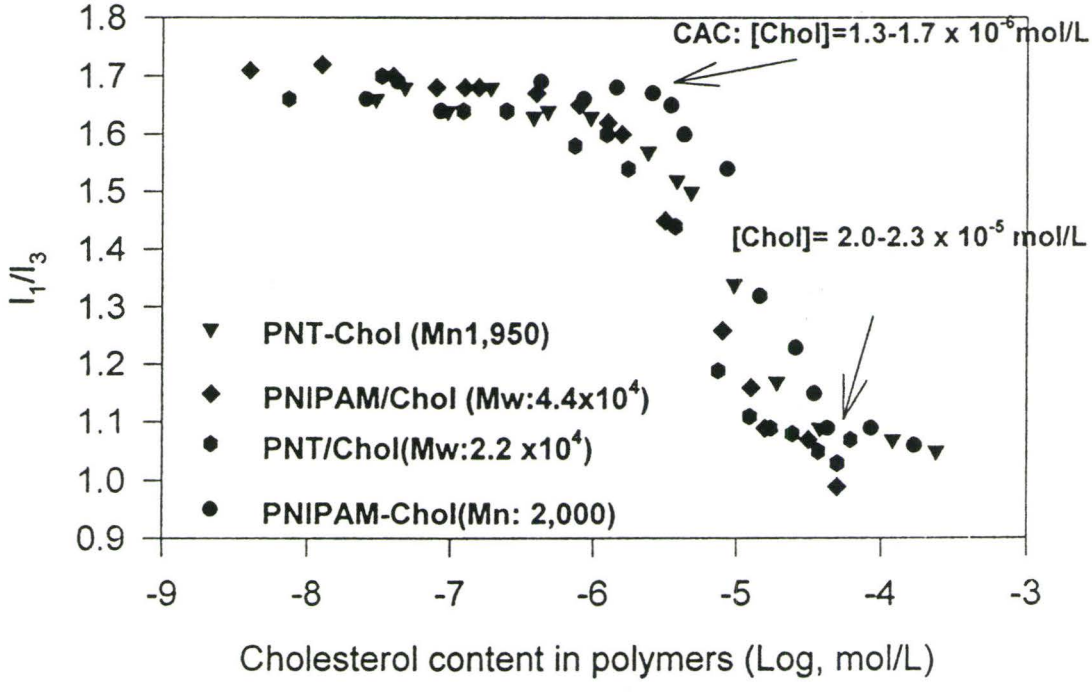


Figure 3.6 I_1/I_3 of HM-oligomers and HM-polymers as a function of cholesterol content (mol/L)

3.6.2 Pyrene labeled cholesterol bearing polymers

Pyrene and cholesterol groups were randomly grafted on the PNIPAM/Py/Chol and PNIPAM/NTHFAM/Py/Chol chain. The emission and excitation spectra of both labeled polymers in both water and methanol were measured at 25°C.

PNIPAM/Py/Chol (Mw $4.4x10^4$)

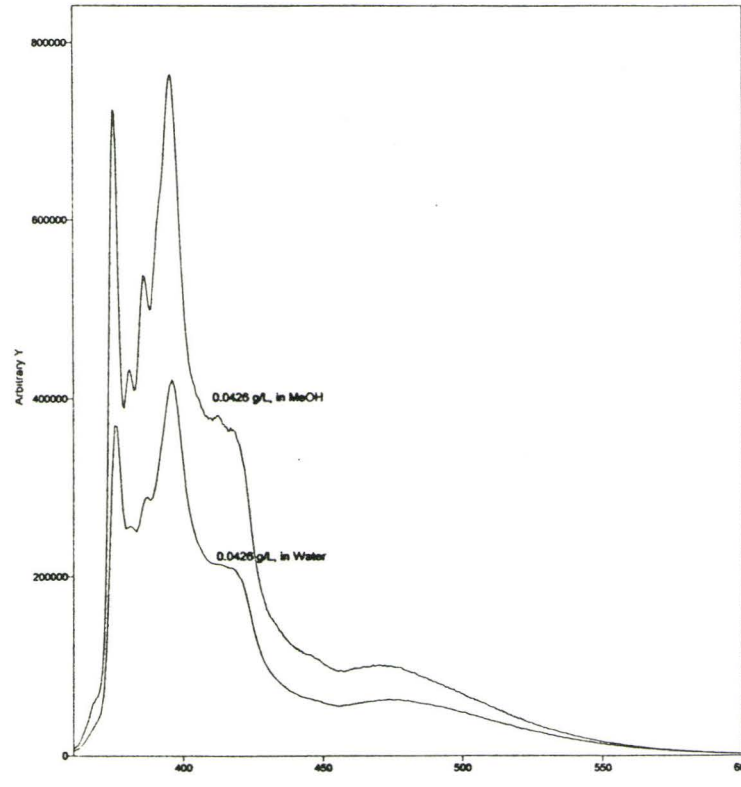


Figure 3.7 Emission spectra of PNIPAM/Py/Chol in Water and in MeOH at 25°C Polymer concentration=0.04 g/L, Excitation wavelength = 344 nm in water And 342 nm in MeOH

Fluorescence emission spectra of PNIPAM/Py/Chol in water and in MeOH are shown in Figure 3.7. Both solutions exhibited pyrene monomer emissions (intensity I_M) and excimer emissions (intensity I_M). The sharp peaks between 360-400 nm originate from pyrene monomer emission and the broad peak centered at ca. 480 nm is due to pyrene excimer emission. Table 3.12 lists the photophysical parameters of PNIPAM/Py/Chol in both water and methanol solution. In this table, I_E/I_M is the ratio of intensities of the excimer emission and the monomer emission (described in section **2.3.5.6**); λ_E is the wavelength of the maximum of excimer emission; and all the parameters from the excitation spectra were briefly introduced in section **1.3.2**.

Table 3. 12 Photophysical parameters of PNIPAM/PY/CHOL in solution*

Parameter	PNIPAM/Py/Chol	
	Water	Methanol
Emission Spectra		
I_E/I_M	0.158	0.137
λ_E, nm	476	473
Excitation Spectra		
P_M	2.70	2.96
P_E	1.75	2.36
$\Delta\lambda$, nm	2.50	
Absorption Spectra		
P^{**}_A	1.46	1.96

*Polymer concentration: 0.0426 g/L.
**peak to valley ratio for the (0,0) transition in absorption.

Polymers take an expanded conformation in a good solvent. As a result, the pyrenes are separated from each other. This gives rise to a large monomer emission and a relatively small excimer emission. Therefore, changes of I_E/I_M can be used to examine the behavior of a polymer in solution. The emission spectrum of the labeled PNIPAM in MeOH shows a much stronger monomer emission with a lower value of I_E/I_M than that measured for polymer in water, which is in agreement with the fact that methanol is a better solvent for PNIPAM/Py/Chol than water.

Table 3.13 The physical properties of PNIPAM/Py/Chol and PNIPAM-C_{18}-Py

HM polymers in aqueous solution	Mw[a] x 10^4	M:HM group[b]: pyrene[c]	I_E/I_M
PNIPAM/Py/Chol (0.1 g/L)	4.4	186:2.3:1.2	0.18
PNIPAM-C_{18}-PY (0.1 g/L)	5.5	186: 1.4:1	0.35

[a] by GPC (against PS) [b] by ^{1}H NMR [c] by UV.

In aqueous solution the ratio of the excimer to monomer emission intensities ($I_E/I_M \approx 0.18$) remained relatively low, however, compared to PNIPAM-C_{18}PY/200 ($I_E/I_M = 0.35$)[34] , which may be attributed to a lower level of HM group and pyrene (in Table 3.13). Probably this was because the attached cholesterol group is bulky and rigid, unlike the relatively flexible alkyl chain, proventing the polymer to adopt a proper conformation for the pyrene labels to form excimers. Also the sterically hindered bulky groups may provid a "shield " between pyrene labels. Thus the "steric effect" limits the amount of excimer formation in this system.

Excitation spectra from PNIPAM/Py/Chol in water and in MeOH offered convincing evidence for ground-state interaction of pyrenes. The differences in spectra of monomer and excimer in aqueous solution: the red shifted spectra ($\Delta\lambda$=2.5), the smaller P_E and the broadened bands for excimer all indicated the preassociation of pyrene labels in water before excitation.

To distinguish whether pyrene aggregates were formed intramolecularly within the same polymer chain or intermolecularly between the different polymer chains, I_E/I_M was monitored as a function of polymer concentration or cholesterol content (Figure 3.8). The ratio I_E/I_M increased with increasing of polymer concentration, indicating the presence of interpolymeric aggregations at higher concentration. A constant ratio of I_E/I_M (ca. 0.11) was obtained for polymer concentration lower than 0.01g/L (CAC) (or cholesterol content ca. 1.30×10^{-6} mol/L), indicating that only intramolecular interaction existed at low concentration. At higher polymer concentration (>0.01 g/L), both inter and intramolecular interactions existed in aqueous solution.

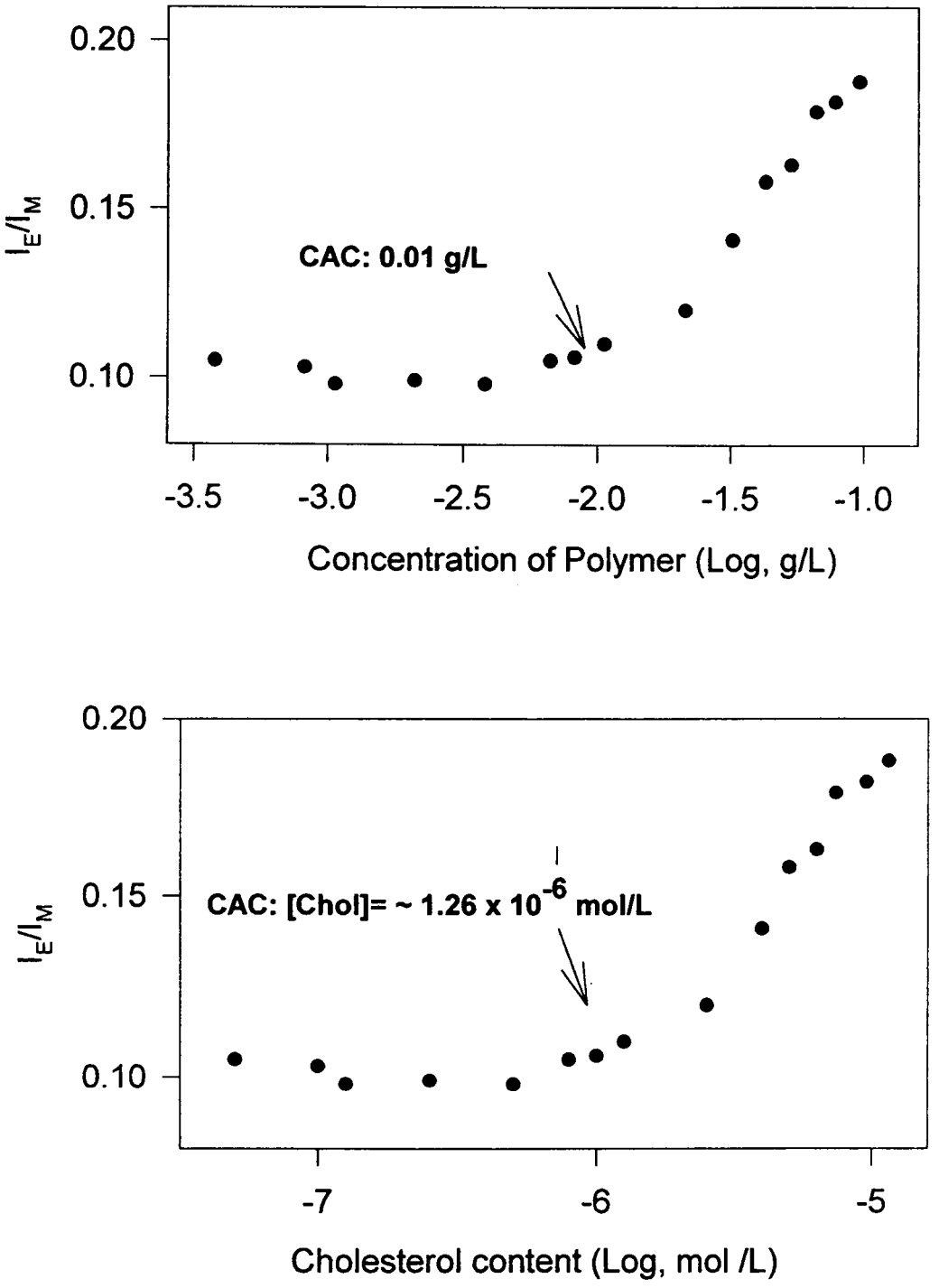


Figure 3.8 I_E/I_M as a function of polymer concentration (top) and cholesterol content (bottom)

The effect of temperature on I_E/I_M for PNIPAM/NTHFAM/Py/Chol aqueous solution (~0.04 g/L) is plotted in Figure 3.9. The ratio I_E/I_M underwent several changes during heating. It reached a maximum at ca. 29°C and then decreased abruptly to a low value of 0.05 at 35°C indicating that below the LCST the pyrenes are in the environment of the hydrophobic microdomains. At the LCST pyrene aggregates were disrupted by the precipitation of polymer from water (the collapse of polymer chains). Above the LCST pyrene excimer emission was very low and almost negligible, indicating that isolated pyrene groups were dissolved in the polymeric phase.

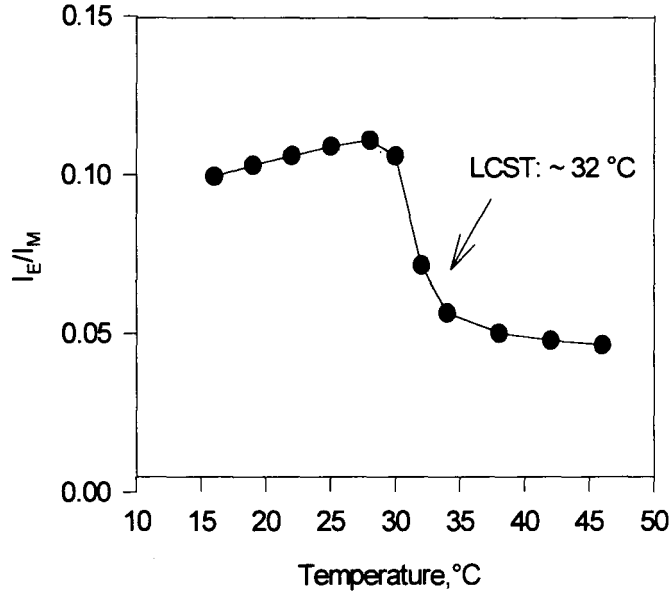


Figure 3.9 The temperature influence on the ratio of I_E/I_M of PNIPAM/Py/Chol aqueous solution (0.04 g/L)

PNIPAM/NHTFAM/Py/Chol

The emission spectra of PNIPAM/NTHFAM/Py/Chol in water and in methanol are shown in Figure 3.10. The intensity of monomer emission in MeOH is stronger with a lower value of I_E/I_M than that measured for the aqueous solution. Compared to the case of PNIPAM/py/Chol (in Figure 3.7), the difference of the intensity of monomer emissions in MeOH and in water is not significant, since this polymer consists of both NIPAM and NTHFAM. The tetrahydrofurfuryl group on the polymer chain formed hydrogen bonds with water molecules. Although, PNIPAM/NTHFAM/Py/Chol was modified with pyrene and cholesterol derivative 2 (ca. 2%), the polymer backbones are very hydrophilic and expanded in water. This result is in agreement with the phenomenon that PNIPAM/NTHFAM/Py/Chol has a higher solubility in water than PNIPAM/Py/Chol does, though methanol still a better solvent for this polymer than water. The excimer emission was observed, which indicated the formation of excimers, but the changing of I_E/I_M as function of polymer concentration was very small. This indicates the lack of intermolecular interaction due to the well-dissolved polymer in water, as we have seen a weak signal from the DLS measurement (Table 3.10). The CAC of this polymer could not be determined from the change in I_E/I_M with polymer concentrations.

Table 3.14 lists the photophysical parameters of PNIPAM/NTHFAM/Py/Chol in water and methanol solution. The differences in spectra of monomer and excimer in water solution (positive $\Delta\lambda$, smaller P_E and broadened excitation spectrum at excimer emission wavelength) provided solid evidence for excimer and indicated that those

pyrene dimers or higher aggregates existed in PNIPAM/NTHFAM/Py/Chol aqueous solution before excitation.

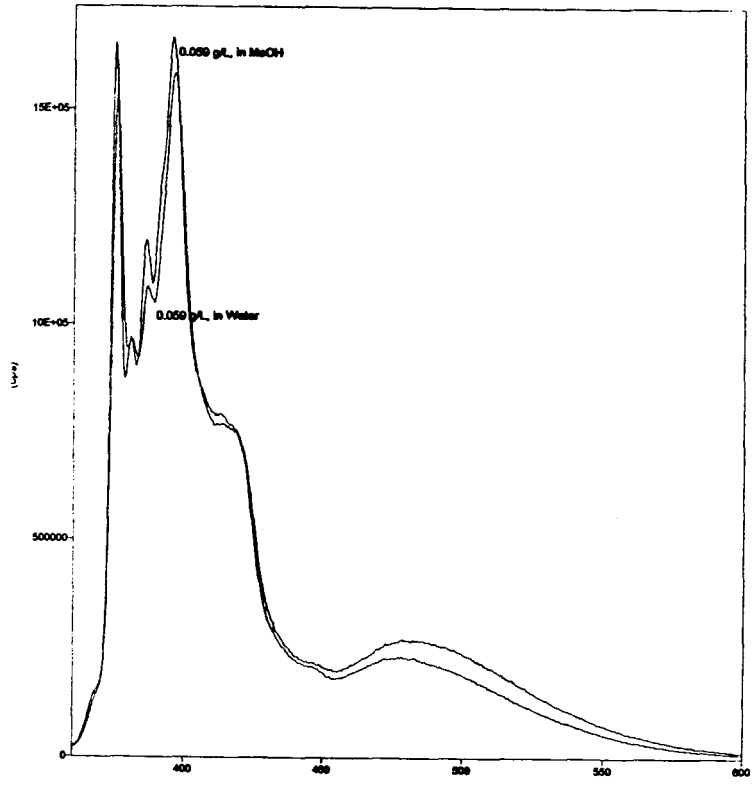


Figure 3.10 Emission spectra of PNIPAM/NTHFAN/Py/Chol in water and in MeOH at 25°C Polymer concentration=0.059 g/L, Excitation wavelength = 344 nm in water and 342 nm in MeOH

Table 3.14 Photophysical parameters of PNIPAM/NTHFAM/Py/Chol in solution*

Parameter	PNIPAM/NTHFAM/Py/Chol	
	Water	Methanol
Emission Spectra		
I_E/I_M	0.177	0.140
λ_E, nm	478.5	480
Excitation Spectra		
P_M	2.38	2.53
P_E	1.81	1.82
$\Delta\lambda$, nm	2.60	
Absorption Spectra		
P^{**}_A	2.96	2.88

*Polymer concentration: 0.059 g/L.

**peak to valley ratio for the (0,0) transition in absorption.

3.6.3 The information from the fluorescence study of both labeled and unlabeled HM-oligomers and HM-polymers

We determined the CACs for the labeled HM-polymer, PNIPAM/Py/Chol (polymer concentration ca. 0.01 g/L, corresponding to cholesterol content ca. 1.30 x 10^{-6} mol/L), not for PNIPAM/NTHFAM/Py/Chol. It is interesting to note that the CAC of the pyrene labeled cholesterol-bearing PNIPAM is similar to the value determined by pyrene probe for unlabeled polymers (Table 3.15). All evidence we obtained from fluorescence confirmed the formation of cholesterol aggregates in aqueous solutions and the leading role of cholesterol group on the onset of micelles in all the polymers we studied here.

Table 3.15 The CACs of HM-oligomers and HM-polymers

HM oligomers or HM polymers	CAC (cholesterol content, mol/L) $\times 10^6$	
	Pyrene label	Pyrene probe
PNIPAM/Py/Chol	1.3 ± 0.5	
PNIPAM/Chol		1.4 ± 0.5
PNIPAM/NTHFAM/Py/Chol*		
PNIPAM/NTHFAM//Chol		1.7 ± 0.5
PNIPAM-Chol		1. 2 ± 0.5
PNIPAM/NTHFAM-Chol		1.5 ± 0.5

* Could not be measured, see text.

As discussed previously, compared to C_{18} group bearing PNIPAM, pyrene labeled cholesterol bearing PNIPAM exhibit a smaller I_E/I_M and unlabeled cholesterol bearing polymers show a lower CAC (cholesterol content) due to the steric hindered cholesterol group. Both cholesterol and C_{18} bearing PNIPAMs have a slightly lower LCST than PNIPAM, resulting from the formation of micellar structure in polymer solutions.

4. Conclusions

Cholesterol bearing PNIPAM and PNIPAM/NTHFAM have been prepared and their solution properties have been studied through several techniques. Among the HM polymers, two types of HM PNIPAM and HM PNIPAM/NTHFAM were made: (1) randomly pyrene labeled or unlabeled cholesterol bearing PNIPAM and PNIPAM/NTHFAM (PNIPAM/Py/Chol, PNIPAM/NTHFAM/Py/Chol, PNIPAM/Chol and PNIPAM/NTHFAM/Chol); (2) cholesterol end capped at one polymer chain end (PNIPAM-Chol and PNIPAM/NTHFAM-Chol).

The LCSTs of all the cholesterol bearing polymers in water are 31-33°C, almost no apparent change compared to those of unmodified PNIPAM (32.5 °C). This was attribute to the formation of micelles in their aqueous solutions. The attached cholesterol groups form the core of the micelle and are insulated from water and therefore made little contribution to the LCST.[30]

Both DLS and fluorescence measurements provided evidence for the formation of micelles in all HM-polymers aqueous solutions. For unlabeled cholesterol bearing polymers (PNIPAM-Chol, PNIPAM/NTHFAM-Chol, PNIPAM/Chol and PNIPAM/NTHFAM/Chol), the CACs (cholesterol content: 1.4-1.7 $\times$ 10^{-6} mol/L) were determined by employing pyrene as probe in fluorescence measurements. The determining fact in guiding micellar formation was shown to be the amount of cholesterol incorporated. The CAC of cholesterol content for PNIPAM is ten times lower than that

of C_{18} for PNIPAM, revealing the much stronger hydrophobic nature of cholesterol group. In the case of pyrene labeled cholesterol bearing polymers (PNIPAM/Py/Chol, PNIPAM/NTHFAM/Py/Chol), the CAC (cholesterol content: 1.3×10^{-6} mol/L) of PNIPAM/Py/Chol was determined by using the ratio of I_E/I_M of pyrene excimer to monomer intensity as a function of polymer concentration. Both pyrene labeled and unlabeled polymers form micelles with a CAC in the same range of cholesterol content. The sterically hindered cholesterol group in the pyrene labeled PNIPAM prevents the neighboring pyrene groups from approaching each other to form excimers, compared to the pyrene labeled C_{18} bearing PNIPAM.

The study of cholesterol bearing PNIPAM and PNIPAM/NTHFAM is a relatively new subject and remains many unanswered questions. A DLS study should be scheduled with a series of polymer concentrations and temperature effect. Copolymerization of NIPAM/NTHFAM can be studied further to investigate the relationship between the polymer chemical composition and its solution properties.

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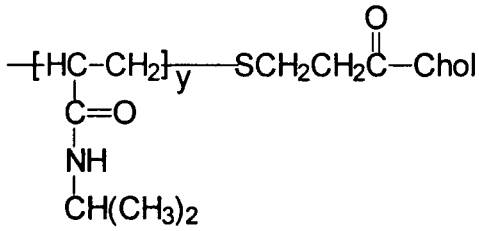
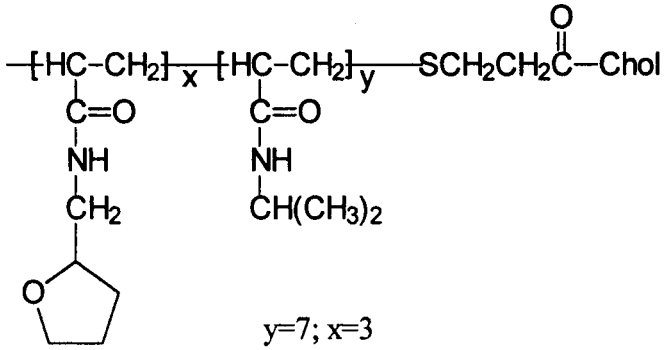
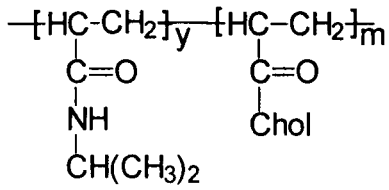
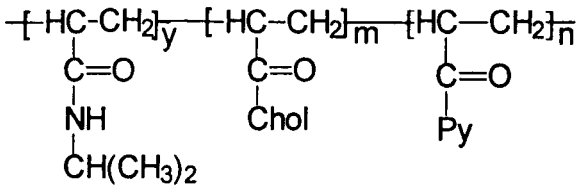
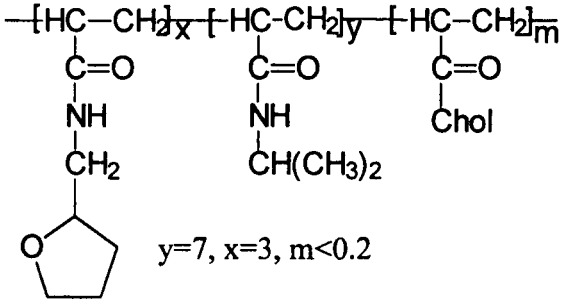
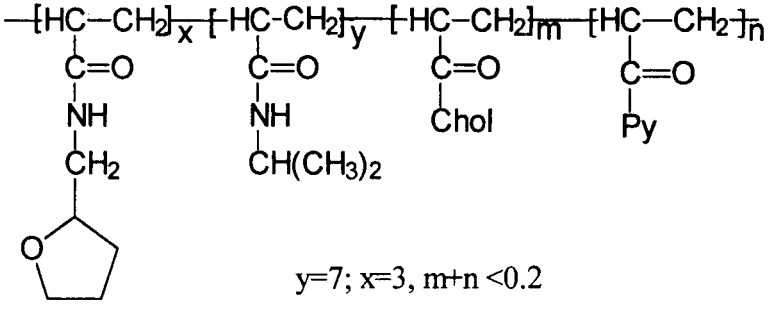
Appendix 1 The Structures of the HM-Polymers Studied in the Thesis

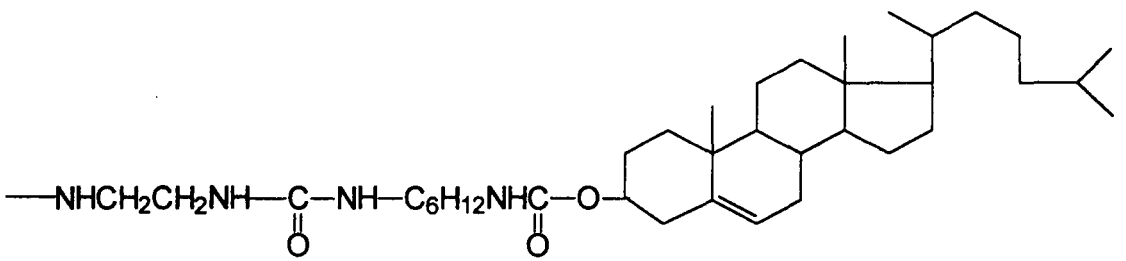
No.	Abbreviation	Structure*
1.1	PNIPAM-Chol	
1.2	PNIPAM/NTHFAM-Chol (PNT-Chol)	 y=7; x=3
1.3	PNIPAM/Chol	 y=100, m<2

Appendix 1 The Structures of the HM-Polymers Studied in the Thesis (*Continued*)

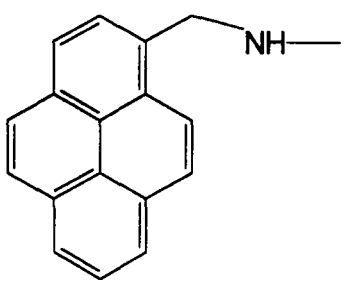
No.	Abbreviation	Structure*
1.4	PNIPAM/Py/Chol	 x=100, m+n < 2
1.5	PNIPAM/NTHFAM/Chol (PNT/Chol)	 y=7, x=3, m<0.2
1.6	PNIPAM/NTHFAM/Py/Chol (PNT/Py/Chol)	 y=7; x=3, m+n <0.2

Appendix 1 The Structures of the HM-Polymers Studied in the Thesis (*Continued*)

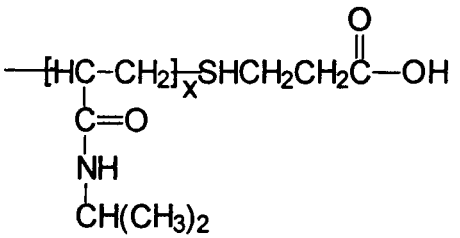
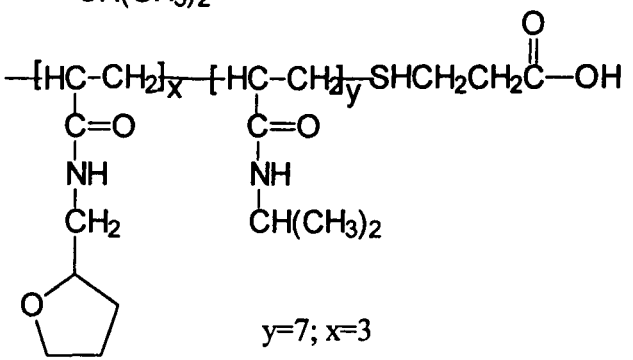
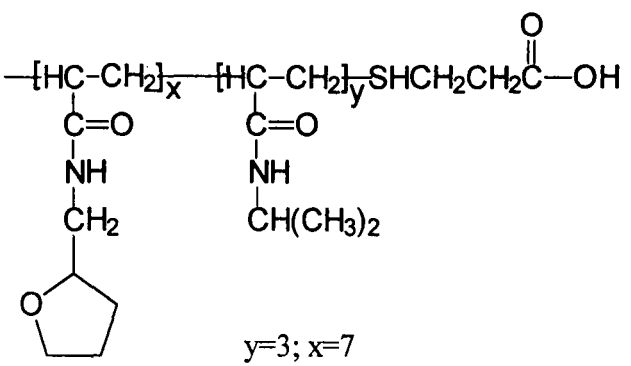
*** Chol:**



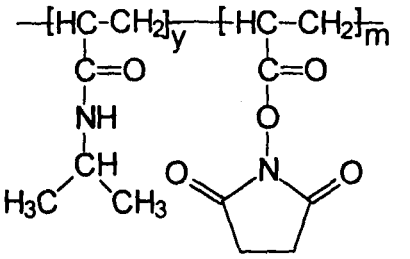
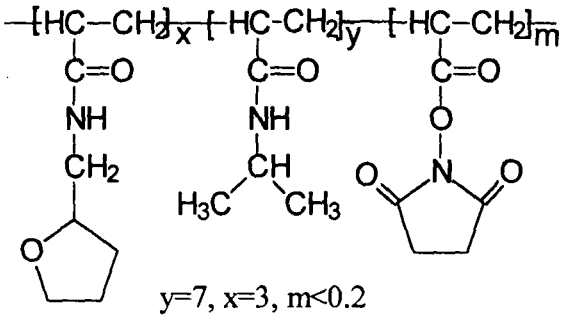
Py:



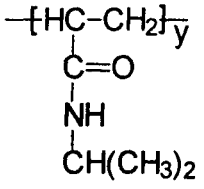
Appendix 2 The Structures of the Intermediates in the Thesis

No.	Abbreviations	Structures
2.1	PNIPAM-COOH	
2.2	PNIPAM/NTHFAM-COOH (PNT-COOH)	 y=7; x=3
2.3	PN_3T_7-COOH	 y=3; x=7

Appendix 2 The Structures of the Intermediates in the Thesis (*Continued)*

No.	Abbreviations	Structures
2.4	PNIPAM/NASI	 y=100, m=2
2.5	PNIPAM/NTHFAM/NASI	 y=7, x=3, m<0.2

Appendix 2 The Structures of the Intermediates in the Thesis (*Continued)*

No.	Abbreviations	Structures
2.6	PNIPAM	
2.7	PNTHFAM