Photosensitive Azo-polysiloxanes for Drug Delivery Applications

SILVIA GRAMA, IOANA-ANDREEA MOLEAVIN, ANCA HODOROG-RUSU, NICOLAE HURDUC, IRINA PRISACARU*, CONSTANTA IBANESCU*

„Gheorghe Asachi" Technical University of Iasi, Department of Natural and Synthetic Polymers, 73 Prof. Dimitrie Mangeron Str., 700050-Iasi, Romania

Photosensitive polymers based on polysiloxanes substituted with different azo and quaternary ammonium groups were synthesized and characterized. The chemical structure of the synthesized polymers was confirmed using ^{1}H-NMR spectroscopy. The photochromic behaviour was evaluated as a result of the interaction with UV light. The micellar aggregation/disaggregation capacity of the polymers was investigated using fluorescence spectroscopy and dynamic light scattering techniques. All the synthesized amphiphilic polymers are able to generate micelles, the critical concentration of aggregation values being situated in the domain (3-13) x 10^{-3} g/L. The morphology of the micellar aggregates is fundamentally influenced by the polymer chemical structure. If in the case of azobenzene and azo-5-crwon-15-ether groups it is possible to obtain 35-155 nm diameter individual micelles, for the azo-6-crown-18-ether group, only small aggregates with diameters below 10 nm can be obtained. The micelle's disaggregation capacity as a consequence of UV irradiation is strongly affected by the hydrophilic/hydrophobic ratio, as well as the chemical structure of the azo groups.

Keywords: azobenzene, polysiloxane, photosensitive micelles

Synthetic polymers, designed in many cases based on principles valuable for biopolymers, often contain a wide variety of functional groups which are necessary to fulfill requirements imposed by particular types of industrial or scientific applications. There is a class of polymers with special chemical properties, called stimuli-responsive polymers [1], smart polymers [2] or environmental-sensitive polymers [3-5]. The main characteristic of smart polymers is their ability to respond to very small changes in specific environmental parameters. The particularity of these materials derives both from the fast macroscopic changes occurring in their structure and the reversibility of these transitions. The response mechanism is usually based on one of the two options: responsive polymers have functional groups sensitive to one or more stimuli or form structures that can be assembled or disassembled by the action of external stimulus [6, 7]. Using light as an external stimulus is particularly interesting because it provides several advantages such as limited chemical contamination and simplicity in manipulation. Compared with other types of stimuli (pH, temperature, redox reactions, magnetic or electric field, ionic factors), it is desirable to use light radiations as external excitants because they allow precise temporal (when the light source is on) and spatial control (if the light is directed to an area) [8]. Light sensitive polymers are functionalized with photo-sensitive groups such as azobenzene, spirobenzopyrane, triphenylmethane, which may undergo reversible structural changes under the action of UV-Vis radiations [9].

Conformational photo-control capacity of the macromolecular chains induced by azobenzene is focused on trans / cis geometrical modifications of the azo group, as a result of the interaction with UV light. If the azobenzene chromophores are linked on the polymeric chain the photo-isomerization process generates conformational changes at the entire chain level, which, in its turn, lead to macroscopic variations in the chemical and physical material properties. This particular behaviour finds a large number of specific applications ranging from electronics to medicine [10-15]. The conformational photo-control capacity of azobenzene derivatives can be applied as principle in drug delivery systems [16]. Thus, in aqueous solutions, amphiphilic azo-polymers self-assemble into spherical micelles, that can be disassembled by the action of UV irradiation.

Usually, the literature reports micellar systems based on surfactants or block copolymers [17-23]. Early research on obtaining polymeric micelles composed of amphiphilic block copolymers containing mesogenic azobenzene groups capable to generate micellar aggregates in water was performed by Zhao et al.; moreover, when exposed to UV-Vis light radiation, reversible morphological changes of the micelles appeared [24-26]. The statistical distribution of the hydrophilic and hydrophobic segments along the polymeric main chain has not received much attention in the literature [27-29].

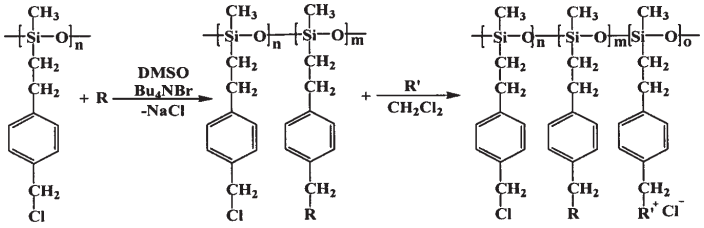
The present paper reports the possibility to obtain UV light sensitive amphiphilic systems with the hydrophilic and hydrophobic segments connected on the same polysiloxanic chain. Different azo groups were used as simultaneously hydrophobic and light sensitive segments, while the quaternary ammonium groups were selected as hydrophilic segments. The polymers were characterized using ^{1}H-NMR and UV-Vis spectroscopy and the micellar aggregation capacity was evaluated using fluorescence spectroscopy and dynamic light scattering (DLS) method. The majority of the synthesized polymers present aggregation capacity, excepting the samples containing azo-6-crown-18-ether groups. The disaggregation capacity of the micelles, induced by the interaction with UV light, was evaluated as well.

Experimental part

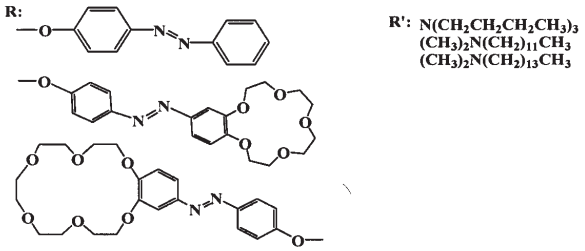
Materials and Methods

Except for the 4'-hydroxyphenylazo-benzo-15-crown-5-ether (15-AEC) and 4'-hydroxy-phenylazo-benzo-18-crown-

email: ibanescu@ch.tuiasi.ro; irina.prisacaru@yahoo.com



Scheme 1 Reaction scheme of the amphiphilic azo-polysiloxanes synthesis



6-ether (18-AEC), all the chemicals were purchased from Aldrich and used without supplementary purification. The hydroxyl-phenylazo-benzo-crown-ethers have been obtained by diazotization of 4'-aminobenzo-15-crown-5-ether, 4'-aminobenzo-18-crown-6-ether respectively and then coupling of diazonium salt to phenol, as mentioned in a previous paper [27].

The amphiphilic polymers were obtained in a two steps process, starting from polysiloxanes containing chlorobenzyl side groups. In the first step, the polysiloxane chain was modified with azo-benzene (25÷65%) or azo-crown-ether groups (25÷55%) by a nucleophilic substitution reaction and in the second step the unreacted chlorobenzyl groups were quaternized using different tertiary amines (scheme 1). Details concerning the synthesis procedure were previously reported [27].

The synthesized polymers were characterized by ^{1}H-NMR, UV and fluorescence spectroscopy and dynamic light scattering (DLS). The ^{1}H-NMR spectra were recorded in $CDCl_3$ on a Brucker Avance DRX 400 MHz spectrometer.

The photochromic properties were evaluated using a UV-1700 Shimadzu spectrophotometer in the range 700÷200 nm. Aqueous polymer solutions have been used to study the photoisomerization phenomena. Irradiation of the solution was performed with a UV lamp (100 W) equipped with 350 nm filter, at room temperature (20÷22°C). The photoisomerization kinetic was calculated based on the UV-VIS spectrum absorption band corresponding to the *trans*-isomer, situated at 345 nm for the azobenzene and 367 nm respectively for crown-ether-azobenzene.

The micellar aggregation capacity was evaluated using the classical method of pyrene fluorescence spectroscopy [28]. Emission spectra were recorded using a Shimadzu RF-5301PC apparatus (λ_{ex} = 332 nm) in the range of 350 ÷ 600 nm, the slots diameter being 5.0 nm for excitation and 3.0 nm for emission. In order to calculate the critical concentration of aggregation (CCA) value, the first (named I_1) and the third (named I_3) absorption peaks corresponding to the fluorescence emission spectrum of pyrene were used. For the free pyrene in water the I_1/I_3 ratio value is around 1.70-1.75. In the presence of an amphiphilic molecule, the I_1/I_3 ratio value decreases due to the pyrene incorporation inside the micelle. The CCA value is considered as the inflection point of the plot I_1/I_3 as a function of concentration.

DLS measurements were performed using a Zetasizer Nano ZS Series at an angle of 173° and equipped with a laser having a wavelength of λ = 633 nm. The micelles were prepared according to the co-solvent method: 10 mg of polymer was dissolved in 1 mL organic solvent (THF) and then 9 mL of water were added drop-by-drop. The final solution concentration was 1 mg/mL. The samples were kept under magnetic stirring for 7÷8 h at room temperature (22°C).

Results and discussions

The amphiphilic azo-polymers were obtained starting from linear polysiloxanes containing chlorobenzyl groups in the side chain. The polysiloxane modification takes place in two steps, the first one supposing the azo groups' connection to the polysiloxanic chain, and the second one the quaternization of the unreacted chlorobenzyl groups (scheme 1). Details concerning the synthesis procedure were previously reported [27]. Some characteristics of the obtained polymers are presented in table 1.

The polymers' chemical structure was confirmed by ^{1}H-NMR analysis. A typical example is presented in figure 1. Details concerning the presence of the characteristic ^{1}H-NMR signal were previously presented [27]. The polymer substitution degree was calculated taking into consideration the signals corresponding to the methylenic chlorobenzyl groups before the reaction (4.5 ppm) reported to the signals that appear after the azo-groups connection (-C_6H_4-**CH_2**-O-C_6H_4-) at 5.0 ppm. Due to the fact that both methylenic groups corresponding to azobenzene and quaternary ammonium segments (-C_6H_4-**CH_2**-O-C_6H_4- and –C_6H_4-**CH_2**-N^+-) present similar chemical shifts (5 ppm), it is compulsory to calculate the substitution degree after the first reaction step and the second one respectively (as one can see in fig.1). The polymers molecular weights (Mn) were also calculated based on ^{1}H-NMR spectra taking into account the starting polymerization degree of the polysiloxane (the linear polysiloxane has for all the samples a molecular weight value Mn = 5,000÷5,500). The polymers containing azobenzene groups present a good thermal stability, the primary degradation processes starting above 250 °C [29].

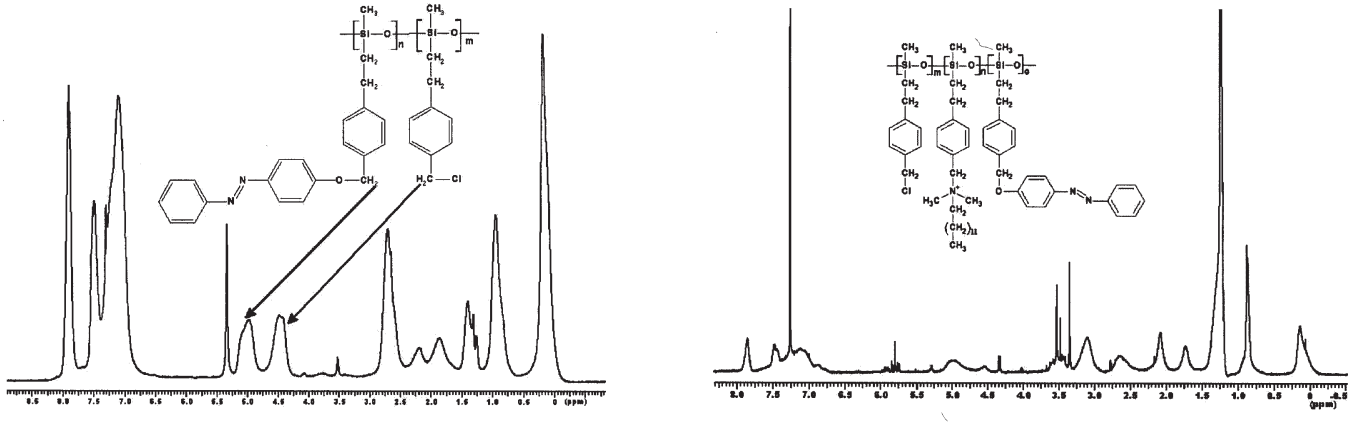
A first step of our studies was meant to evaluate the photoisomerization capacity of the amphiphilic polymers in water. This aspect is very important for establishing the maximum content of *cis*-isomer, as a function of the UV-irradiation time. Our previous studies evidenced that in chloroform solution the azo-polysiloxanes containing ammonium groups have a good response to light stimuli, 10 to 20 seconds of irradiation being enough to obtain the maximum content in *cis*-isomer (around 80%) [30, 31]. As one can see in figure 2 the amphiphilic azo-polymers have different behaviors as a function of the azo group structure.

If in the case of the azobenzene group a maximum conversion degree in *cis* isomer situated around 67% can be obtained, in the case of azo-crown-ethers, only a maximum value of 35% can be attained. The photochromic equilibrium is not influenced by the amine structure, but the photoisomerization rate is affected. The

Sample	Azo group		Tertiary amine		M_n	CCA ($g\ L^{-1}$)
	Type	Content (%)	Type	Content (%)		
1	azobenzene	50	TBA	31	8800	3×10^{-3}
2	azobenzene	30	TBA	41	8450	10×10^{-3}
3	azobenzene	60	DMDA	25	9000	6×10^{-3}
4	azobenzene	45	DMDA	24	8600	6×10^{-3}
6	azobenzene	30	DMDA	50	9400	9×10^{-3}
7	azobenzene	28	DMDA	19	7400	10×10^{-3}
8	azobenzene	55	DMTDA	25	8500	5×10^{-3}
9	azobenzene	34	DMTDA	24	7800	6×10^{-3}
10	azobenzene	25	DMTDA	52	7950	10×10^{-3}
11	15-ACE	45	TBA	37	10900	7×10^{-3}
12	15-ACE	28	TBA	22	8800	10×10^{-3}
13	18-ACE	53	TBA	15	11950	10×10^{-3}
14	15-ACE	45	DMTDA	39	11600	4×10^{-3}
15	18-ACE	36	DMDTA	46	11700	13×10^{-3}

TBA = tributhylamine; DMDA= dimethyldodecylamine; DMTDA= dimethyltetradecylamine

Table 1
CHARACTERISTICS OF THE AMPHIPHILIC AZO-POLYMERS



(A) (B)

Fig. 1 ^{1}H-NMR typical spectra for (A) azo-polysiloxane and (B) azo-polysiloxane quaternized with dimethyldodecylamine (Sample 6)

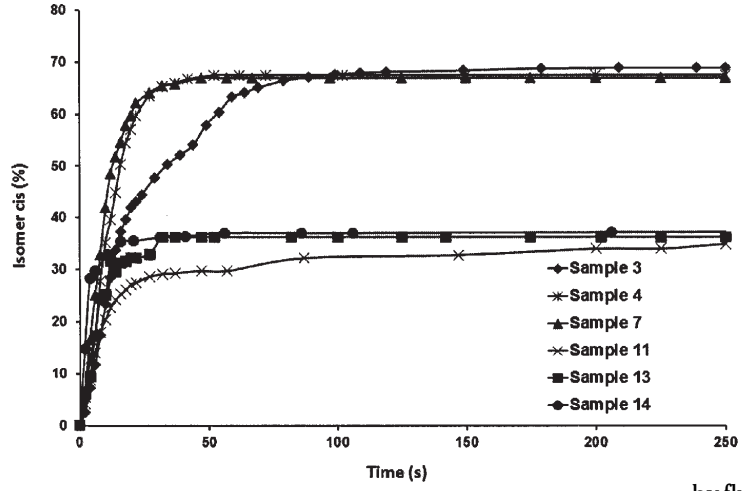


Fig.2 Photoisomerization kinetic curves corresponding to Samples 3, 4, 7, 11, 13 and 14

UV irradiation time necessary to obtain the maximum conversion degree in *cis*-isomer is comparable for the aqueous and chloroform solutions.

The next step in our study was to evaluate the micellar aggregation capacity of the amphiphilic azo-polysiloxanes by fluorescence spectroscopy. The classical method based on pyrene was used, the spectral studies leading to the identification of the critical concentrations of micellar aggregation (CCA) using pyrene as a standard. Typical examples concerning polymers aggregation behavior and

evaluation of the CCA values are presented in figure 4. The CCA values were considered as the inflexion point from the curves that represent the plot of the I_1/I_3 ratio as a function of the polymer solution concentration in logarithmic scale. The CCA values for the synthesized polymers are given in table 1. It was found that all the polymers are able to generate micelles, CCA values being situated between 2 x 10^{-3} g / L and 13 x 10^{-3} g / L. The values are lower compared to amphiphilic small molecules. This is probable due to the high aggregation capacity of the azobenzene groups inside the micelles.

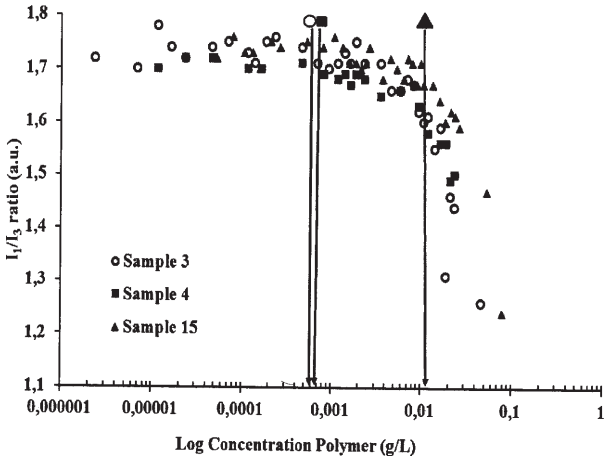


Fig. 3. Plot of I_1/I_3 ratio as a function of azo-polysiloxane concentration corresponding to Samples 3, 4 and 15

Another objective of the present study was to evidence if the micellar aggregates respect the rules for drug encapsulation and release. As a consequence, DLS studies were performed, obtaining information about the size and type/morphology of the micelles function of their chemical structure and the hydrophilic/hydrophobic ratio. In agreement with our previous studies using amines with long aliphatic segments (like DMDA), inter-micellar association phenomena are likely to appear, as in the case of Sample 3 [27]. But, for the drug delivery systems, individual micelles with diameters around 100 nm are preferred [32-34].

The DLS measurements were achieved at two different concentrations: 0.1 and 0.05 mg/mL. As presented in figure 4, for Sample 4 (0.1 mg/mL) the average diameter is situated at 66 nm and the value of the polydispersity index is 0.139, reasonable values for drug delivery systems. Increasing the quantity of the hydrophilic segments (Sample 6) for the same concentration (0.1 mg/mL) the micelles average diameter is only 36 nm being situated below optimum values. But modifying the hydrophilic/hydrophobic ratio and the total substitution degree (Sample 7) the micelles diameter increases up to 155 nm, having a very small value of the polydispersity index (0.032). In this case, no inter-micellar aggregation takes place, probably due to the small amount of quaternary ammonium groups (17%). The inter-molecular aggregation process characteristic for Sample 3 is very limited for Samples 4 and 7, capable to generate individual micelles due to a good control of the hydrophilic/hydrophobic ratio. On the other hand, the increase of the hydrophilic contribution leads to the aggregation of a small number of polymeric chains which probably generate only primary aggregates, inadequate for the encapsulation and controlled release of drugs.

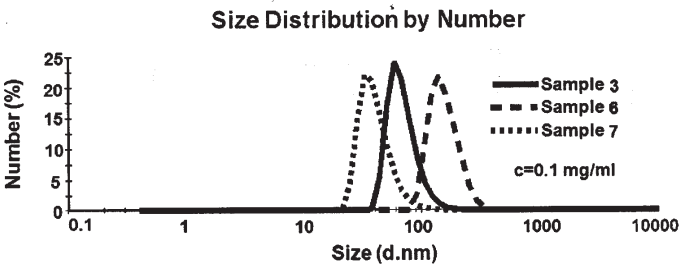


Fig. 4. DLS curves corresponding to Samples 3, 6 and 7

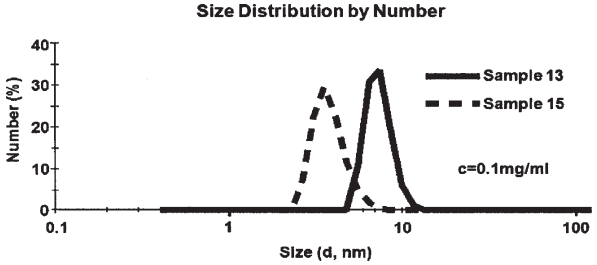


Fig. 5. DLS curves corresponding to Samples 13 and 15

DLS studies were also carried out for polysiloxanes functionalized with azo-crown-ethers and quaternary ammonium groups, at the same concentrations. In the case of Sample 12, due to the low amount of azo groups, micelles with a diameter around 140 nm are obtained. Modifying the crown-ether dimension (from 15-crown-5 to 18-crown-6), the DLS analysis reveals only the formation of some primary aggregates, with a diameter of 7 nm in the case of sample 13 and 3 nm, respectively for Sample 15 (fig. 5). It is possible that the higher diameter of the 18-crown-6 group prevents the micellar aggregation process.

Finally, the micelles disaggregation capacity as a consequence of UV irradiation was evaluated. The micelles' disaggregation is a result of *trans-cis* photoisomerization process of the azo groups, accomplished by significant geometrical changes. As a function of the hydrophobic/

Sample	CCA (g/L)	I_1/I_3 ratio		
		Non-irradiated	Irradiated 15 min	Irradiated 30 min
3	6 X 10^{-3}	1.35	1.66	1.72
6	9 X 10^{-3}	1.32	1.55	1.61
7	10 x 10^{-3}	1.16	1.53	1.58
12	10 x 10^{-3}	1.41	1.45	1.45
13	10 x10^{-3}	1.28	1.42	1.42
15	13 x 10^{-3}	1.28	1.51	1.52

Table 2
DISAGGREGATION STUDIES OF AZO-POLYSILOXANE MICELLES UNDER UV IRRADIATION

hydrophilic ratio, the *trans-cis* photoisomerization process can generate a partial or a total disaggregation. The micelles response to UV irradiation was investigated using fluorescence spectroscopy, with pyrene as marker. When the micellar aggregates are present in the system, the pyrene is encapsulated inside them, and the I_1/I_3 ratio decreases from 1.7 to 1.2. After the disaggregation process, the encapsulated pyrene is released and as a consequence the I_1/I_3 ratio returns to the starting values (close to 1.7)

The results concerning the micelles' behaviour in the presence of UV light are presented in table 2.

The best results were obtained in the case of Sample 3, after 15 min of UV irradiation (350 nm), the ratio I_1/I_3 having a value (1.66) situated very close to the 1.72 limit. In the case of Samples 6 and 7 good results are obtained, even if the maximum I_1/I_3 ratio value does not reach the limit of 1.72. Not so satisfactory results are obtained in the case of azo-crown-ethers, both concerning the starting and final I_1/I_3 values. These results reflect no disaggregation processes after the UV irradiation, as in the case of Sample 12.

Conclusions

Photo-sensitive polymers based on azo-polysiloxanes modified with quaternary ammonium groups were synthesized and characterized. Two types of azobenzene groups (azophenolate and azo-crown-ethers) and different types of tertiary amines (TBA, DMDA, DMTDA) were used, the interest being focused on obtaining individual photo-sensitive micelles for drug delivery systems. The photochromic response of the amphiphilic systems in aqueous solutions was studied. The polymers responses in water and chloroform were similar, 15-20 s of UV irradiation being enough to obtain the maximum *cis*-azo-isomer content. All the amphiphilic polymers are able to generate micelles, the *CCA* values being situated between 2×10^{-3} g / L and 1.3×10^{-2} g / L. Higher values of *CCA* correspond to amphiphilic systems based on azo-crown ether groups (due to lower content in hydrophobic segments). The DLS studies showed that the morphology of the micellar aggregates is influenced by the hydrophilic / hydrophobic ratio and by the polymer chemical structure. The best results concerning the micelles diameters are obtained in the case of polysiloxanes modified with azobenzene and DMDA. Excepting Sample 12, the amphiphilic polymers based on azo-crown-ether groups form only small dimensions (below 10 nm) primary aggregates. The stability of micelles under UV irradiation for azobenzene groups modified polysiloxanes is strongly affected by the hydrophilic/hydrophobic ratio, as well as the azobenzene content: systems with over 60 % azobenzene content completely disaggregate, while the ones with less than 50 % azobenzene are more stable, allowing only a partial release of pyrene from the inside of the micelle. Systems based on azo-crown-ether groups modified polysiloxanes proved to be inadequate for the controlled immobilization and release because of the defective aggregation mode, as it could be seen from the DLS analysis.

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