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ORIGINAL PAPER

Azo-polymers modified with nucleobases and their interactions with DNA molecules

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Abstract The photo-fluidization process which is specific for azo-materials opens a new perspective for their use in the field of molecules nano manipulation at the surface of the azo polymer films. This is possible considering that in the case of the UV irradiation from a polarized laser source the azo material has an unidirectional flow. Here, we investigated the structuring phenomena occurring on the surface of the azo-polysiloxanes films modified with nucleobases, upon UV irradiation. Measurements of topography and adhesive forces between polymeric substrates and a hydrophilic probe have been done by atomic force microscopy (AFM). The response of the material upon irradiation has been investigated also by using UV−VIS spectroscopy. This method allowed us to draw the photo-isomerization and relaxation curves. Also, preliminary tests were conducted to determine the capacity of the film surface to immobilize DNA molecules.

Keywords AFM · Molecular modeling · Azopolysiloxane · DNA · Nucleobases

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Introduction

The general concept of nanoscience and nanotechnology is gradually shifting from the synthesis of individual components to their association into complex systems capable to self-assembly or respond at molecular level to external stimuli [1–10]. One of the most interesting molecules that respond to light-stimuli is the azobenzene, due to its *trans–cis* reversible photo-isomerization capacity. The isomerization process of azobenzene based materials has very interesting potential applications, such as surface relief gratings (SRGs) for holographic storage and optical switching [11–15], nanomanipulation of molecules [16, 17], controlled drug delivery [18], and mechano-sensitive channels technology [19]. Absorption bands of the azobenzene groups are strongly influenced by the nature of the substituent placed in p, p′-positions. If both absorption bands of the *trans* and *cis* isomers overlap, a continuously photo-induced *trans–cis–trans* cycle takes place during irradiation with a single wavelength [19]. This continuous *trans–cis* isomerization process is believed to give rise to a special transition of the azo-polymer films, from an isotropic solid state to an anisotropic fluid phase (occurring even below the glass transition, T_g). This phenomenon has been previously explained by the "photo-fluidization" and "conformational instability" concepts [16]. This property, specific for azo-materials, opens a new perspective for their use in the field of molecules nano manipulation at the surface of the azo polymer films. This is possible considering that in the case of the UV irradiation from a polarized laser source, the azo material has an unidirectional flow. However, to ensure the stability of the molecules on the film surface during the nanomanipulation, it is necessary to facilitate physical interactions between the substrate and the film. Since the next step is to immobilize and nano-manipulate biomolecules (DNA and proteins) or complex bio-aggregates (such as viruses), we synthesized azo-polymers modified with nucleobases, capable to generate hydrogen bonds at their surface.

The interactions which can be formed between the surface of the azo polymer film and biomolecules are very complex. There have been cases reported in literature, describing azo-polymers which can immobilize a protein on the film surface, even in the absence of the groups capable to generate strong physical interactions [20]. Under these circumstances, a better understanding of the phenomena taking place on the surface of the azo-polymeric film, during the UV irradiation, is essential. Preliminary studies developed by our group [21] proved that once the UV irradiation starts, two distinct phenomena take place on the surface, at two different time scales. The first and fastest one is the *trans–cis* photo-isomerization of azo-benzene groups. The life-time of this stage is in the picoseconds order [22, 23]. This phenomenon occurs together with significant changes of the azo-benzene dipolar momentum. The second phenomenon corresponds to the system reorganization at supramolecular level. The life-time of this stage is in the range of minutes [21]. As a result, the correlation of the phenomena occurring at molecular scale with changes of the azo-polymer film surface properties is a very complex issue.

The aim of this study is to investigate the structuring phenomena occurring on the surface of the azo-polysiloxanes films modified with nucleobases, upon UV irradiation and their interactions with DNA molecules. The re-ordering processes that take place on the film surface were studied using the surface energy values.

Measurements of topography and adhesive forces between polymeric substrates and a hydrophilic probe have been done by atomic force microscopy (AFM). The response of the material upon irradiation has been investigated also by using UV–VIS spectroscopy. This method allowed us to draw the photo-isomerization and relaxation curves. For a better understanding of the macromolecular chains and the substitute groups assembly at the film surface, molecular modeling tests have been done by using Accelrys-Materials Studio 4.0 [24]. Also, preliminary tests were conducted to determine the capacity of the film surface to immobilize DNA molecules and the DNA controlled delivery.

Experimental

The azo-polysiloxanes were obtained in a two-step reaction, starting from a polysiloxane containing chlorobenzyl groups in the side-chain. In the first step, the polysiloxane was modified with 4-hydroxyazobenzene (50–60% substitution degree) and, in the second one, the unreacted chlorobenzyl groups were substituted with nucleobases. Details concerning polymer synthesis and characterization have been previously reported [16]. The azo- and nucleobases-units are statistically distributed along the polymeric chain.

The synthesis reaction scheme is given in Fig. 1.

The azo-polysiloxane films were spin-coated from chloroform solutions onto either a glass plate, 3 wt%, 1500 rpm, for AFM experimentation (thickness around 500 nm), or a quartz cell, by casting 0.5 wt%, for the photochromic studies (film thickness around 500–600 nm). Irradiation of the films was carried out at 365 nm (in the peak of *trans* isomers) with a 100 W mercury lamp (Bioblock model, irradiance of 7 mW/cm^2 at 30 cm from the filter). During the irradiation, the polymeric film was exposed to a compressed air stream in order to maintain a constant temperature of 22 °C. The thermal relaxation of films was achieved in visible light, at 22 °C.

Molecular simulations performed by using the Accelrys software (Materials Studio 4.0), evidenced that, most probably, the nucleobases are placed on the

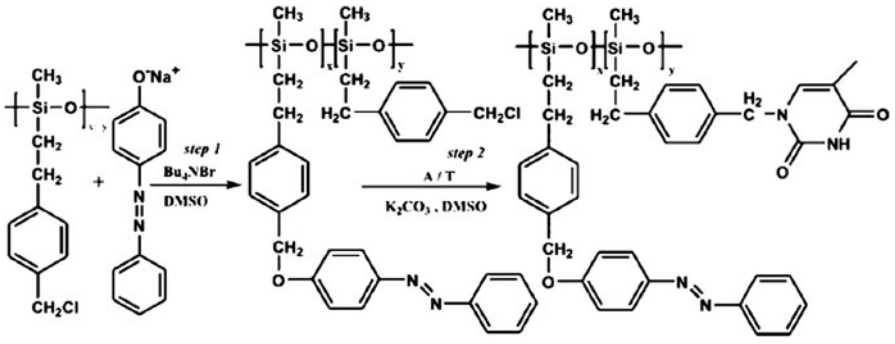

Fig. 1 Polymer synthesis reaction scheme

surface of the film. In the first step, the conformation of an isolated chain was obtained and in the second one an amorphous cell was generated, to anticipate the supramolecular ordering in condensate phase. The single polymeric chain conformation was obtained using a Molecular Mechanic procedure, the Forcite module (Dreiding and PCFF force fields, alternatively with molecular dynamics, to identify the global minimum of the energy value); the investigated single chain had a polymerization degree DP = 20, similar with the results obtained by synthesis. The simulations for the azo-polysiloxanes amorphous cell were performed using the "confined layer" option, the system being minimized after the construction step.

The topography and force spectroscopy experiments were carried out by operating an MFP-3D Asylum Research Atomic Force Microscope in AC mode, in the air and Force Map mode, respectively. The AFM probes were microfabricated as rectangular cantilevers (HA-NC polysilicon ETALON NT-MDT, Russia) with a nominal spring constant of 2 N/m. The flat surfaces used to deposit the polymer film consisted of polished silicon wafers (Silicon Valley Microelectronics, USA).

The DNA immobilization assay was performed using double-stranded (ds) plasmidial DNA (pTriex 1.1). Briefly, the DNA was amplified in *E. coli* cells and purified from 3 mL of culture using the Qiagen kit, according to the manufacturer's instructions. The plasmid was further linearized by EcoRI digestion, gel purified, and re-suspended in TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8) to a final concentration of 1 μg/mL. Although sample, standardized and highly reproducible, plasmid DNA purification is always characterized be agarose gel electrophoresis. The plasmid chosen for this experiment is of medium size (5,301 base pairs) and maintains total integrity following gel purification procedures, thus a homogeneous, single sized DNA sample was applied to the azo-polymers. The films were incubated with this solution for 30 min, at room temperature and the unbound DNA was removed and measured spectrophotometrically (Pharma Biotech Ultrospec 3000 spectrophotometer). The films were washed three times with TE buffer; the bound DNA was released with elution buffer (1.25 M NaCl, 50 mM Tris–HCl, pH 8.5, containing 15% v/v isopropanol) and quantified spectrophotometrically.

Results and discussions

In Table 1 are summarized the main characteristics of the synthesized polymers.

The first stage of our study involved the investigation of the polymer's photochromic behavior, in order to establish the optimal UV irradiation parameters. This step was required to prepare the UV-treated substrates for the next AFM topography and force spectroscopy analysis. This study has surveyed the films response speed upon UV irradiation and also, the relaxation kinetics after irradiation.

In the case of Sample 1 (Fig. 2), it can be observed that the photo-isomerization equilibrium occurs quickly (after 40 s of irradiation), the maximum of *cis* isomer conversion degree being 68%. The relaxation in the presence of the natural light takes place with a relatively similar rate but the total relaxation time is grater (100 s). The films behavior, particularly the relaxation process, is changing upon

Table 1 The characteristics of synthesized polymers

Sample no.	Azo (%)	Nucleobases (%)	Mn	Azo-units content/ polymeric chain[a]	Nucleobase-units content/polymeric chain[a]
1	62	–	6.350	12	–
2	60	13 (Thymine)	6.900	12	2.6
3	55	24 (Thymine)	7.050	11	4.8
4	53	35 (Thymine)	7.250	10.6	7
5	60	17 (Adenine)	6.600	12	3.5
6	59	35 (Adenine)	7.400	12	7

[a] Average polymerization degree of the polysiloxanic chains = 20

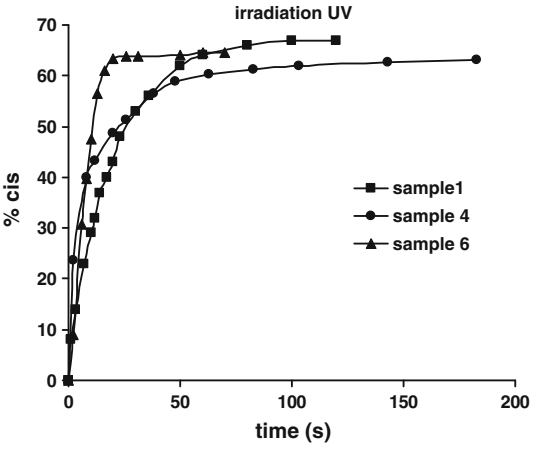

Fig. 2 Polymer response on the UV irradiation corresponding to the Samples 1, 4, and 6

nucleobases connection (along with the azobenzene groups) on the main chains (Figs. 2, 3). If in the case of Sample 1, there are necessary 100 s for the relaxation, in the case of Samples 4 and 6 there are only 10–20 s needed. Upon UV irradiation the material's rate of response remains practically unchanged, the photo-isomerization equilibrium being attained at 60–65% yield for the *cis* isomer. It can be inferred that, from the point of view of the material's response rate at UV/Vis exposure, polymers 4 and 6 are the most suitable for laser nanomanipulation applications in comparison with polymer 1.

Our previous studies indicated that the polymeric matrix responds, too, of the *trans*/*cis* photo isomerization processes. However, the reaction time is different from that of the azo group [21, 25, 26]. Under these circumstances, it is possible that as a result of the polysiloxane matrix reorganization at the supramolecular level, the main-chain migrates from the secondary layer to the interface. Moreover, because of the high flexibility of the main chain, some of the lateral groups may switch their position from the film exterior towards the sub-interfacial layer.

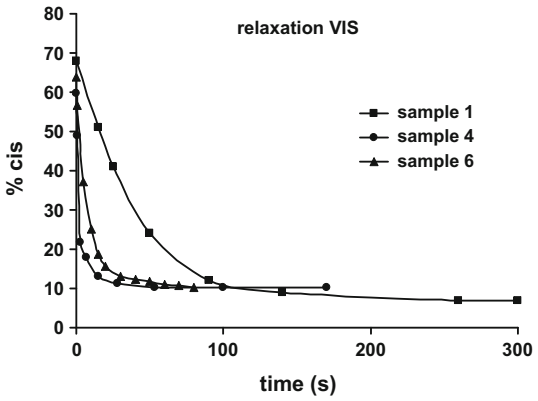

Fig. 3 Polymer relaxation in visible light corresponding to the Samples 1, 4, and 6

The next step in our study was to identify the changes in the magnitude and distribution of adhesion forces recorded between an AFM probe (naturally hydrophilic because of a thin layer of silica) and the exposed and un-exposed films to UV radiation. A number of previously published studies done on polymers with similar structures (without nucleobases in their structure), highlighted that the most important changes on the films interfacial structure happen in the first 30 min from the irradiation [21, 27]. Therefore, we decided to evaluate the adhesion forces half of an hour after the irradiation, meanwhile the samples being kept in the dark.

In the case of the thymine-modified polymers, there are significant changes as a result of the thymine concentration (Figs. 4, 5, 6). In the case of the sample with a reduced content of nucleobases, the surface energy seems to increase, while in the case of the samples with a higher thymine content, adhesion is significantly reduced. Adhesion magnitude changes are a result of chemical groups restructuring process at the interface, induced by changes in the composition, as well as by the azo dipole momentum shift generated by photo-isomerization. Previously [26], it has been proposed that a fraction of the lateral groups could pivot around the main chain, therefore migrating from the outer layer towards the sub-interfacial layer.

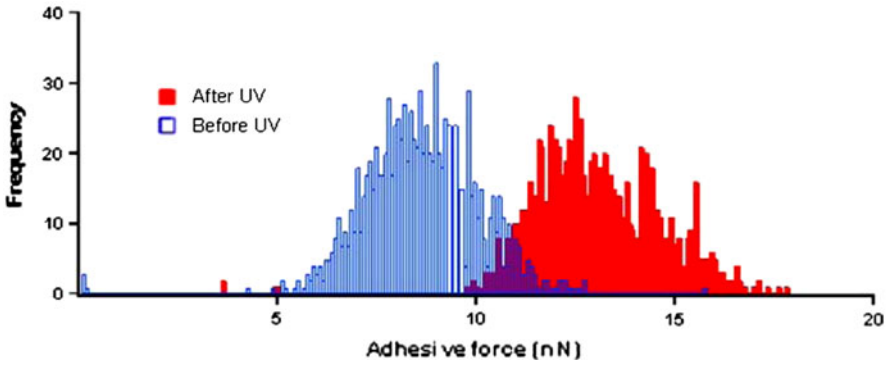

Fig. 4 Adhesive forces corresponding to Sample 2 before and after UV exposure

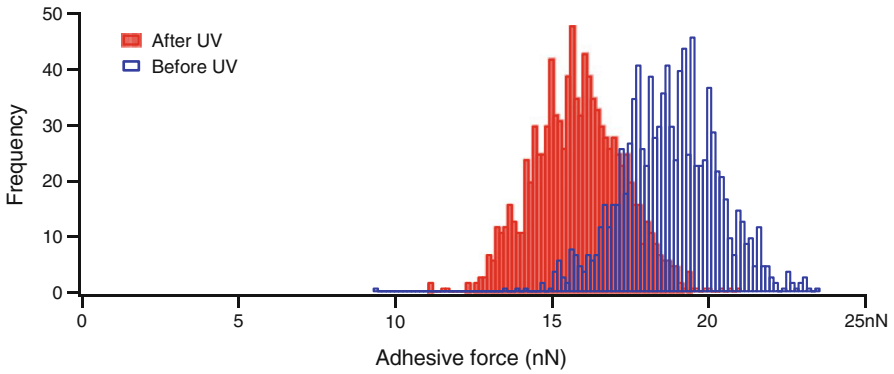

Fig. 5 Adhesive forces corresponding to Sample 3 before and after UV exposure

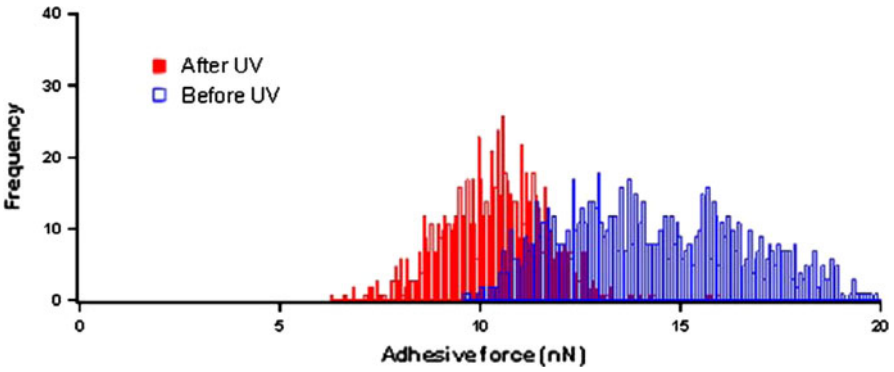

Fig. 6 Adhesive forces corresponding to Sample 4 before and after UV exposure

This process could involve the azo groups, as well as the nucleobases, thus making this system more complicated, and difficult to understand. The reduction of the adhesion could be explained by a potential increase of the density of the polysiloxane chains at the interfacial layer.

In the case of adenine, adhesion is always smaller than before exposure, but this time, the polymer with a lower content of adenine, shows the widest differences in terms of surface energy (Figs. 7, 8). The adhesion force modifications suggest that in the case of a smaller adenine content, much important reorganization processes at the film surface take place. Future experiments with series of concentrations should clarify the rules governing the surface transformations undergoing as a result of UV exposure.

As far as the topography analysis is concerned, the samples preserved their surface profile. Even after the UV exposure, they maintain their RMS roughness below 1 nm.

In addition to the tests described above, experiments were performed to establish the capacity of the nucleobases modified azo-polysiloxane to immobilize dsDNA and allow its release from the film under standard elution conditions [28]. For a

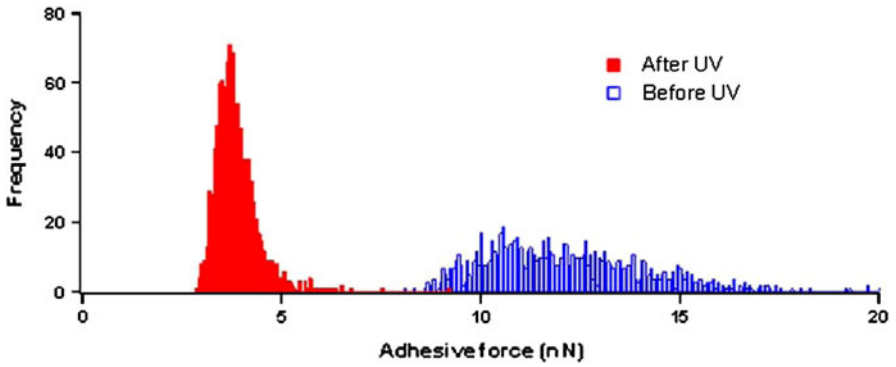

Fig. 7 Adhesive forces corresponding to Sample 5 before and after UV exposure

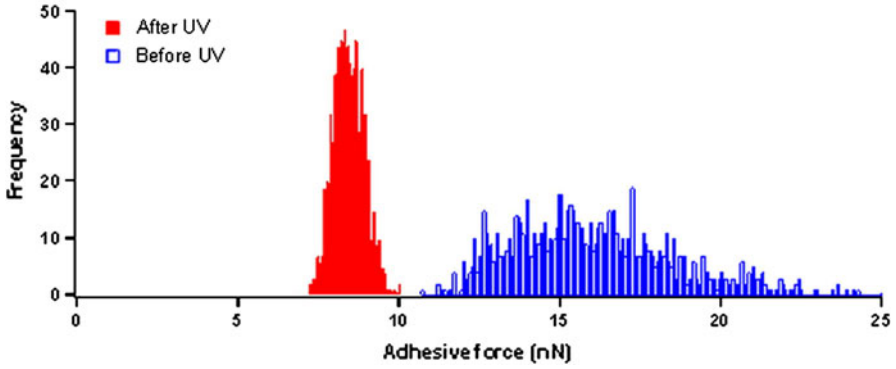

Fig. 8 Adhesive forces corresponding to Sample 6 before and after UV exposure

Table 2 DNA immobilization on the film surfaces

Sample no.	Azo-benzene content (%)	Nucleobases content (%)	% Immobilized DNA (ratio reported to the initial quantity)	% Eluted DNA (ratio reported to the immobilized chains)
7	65	–	0	0
8	60	13 (Thymine)	9.7	0
9	60	17 (Adenine)	34.7	5.8
10	–	87 (Thymine)	3.5	100
11	–	81 (Adenine)	0	0

better understanding of the dsDNA binding/release properties, three types of films have been investigated and compared (Table 2): the polysiloxanes modified with either azo-benzene (Sample 7), the nucleobases-modified azo-polysiloxanes (Samples 8 and 9), and nucleobases only (Samples 10 and 11).

The data in Table 2 show that the polysiloxanes modified with either azo-benzene (Sample 7) or nucleobases (Samples 10 and 11) have a very poor dsDNA immobilization capacity, if at all. In comparison, the presence of nucleobases along with the azo-benzene ensures the binding of a significant amount of dsDNA. This difference could be explained by the azo-benzene preferential position at the film interface. Thus, there is literature data [20] suggesting that the azo groups need to adopt a parallel conformation at the interface, in order to promote interaction with double-helix, dsDNA.

The best results have been obtained in the case of the polysiloxane modified with azobenzene and adenine (Sample 9), which seems to attach the largest amount of DNA. Once immobilized, the DNA is stable enough to sustain variations of the electrolyte concentration. We are currently performing studies of the DNA stability on the film surface upon UV irradiation. Our next research step would be to study interactions between DNA-coated colloids and the substrates investigated here by using AFM.

Preliminary molecular modeling studies have shown that in the case of the polysiloxanes modified with azo-benzene only, the azo groups are oriented perpendicularly to the surface, therefore inhibiting DNA attachment (Fig. 9). Similar results were obtained in the case of Sample 8 (Fig. 10), the azo groups having similar orientation.

A different situation is highlighted in the case of Sample 9, where the azo groups adopt a surface parallel orientation (Fig. 11). These results confirm the hypothesis that the relative position of azo groups can be responsible for the efficiency of DNA immobilization at the surface, which is in agreement with the literature data [20].

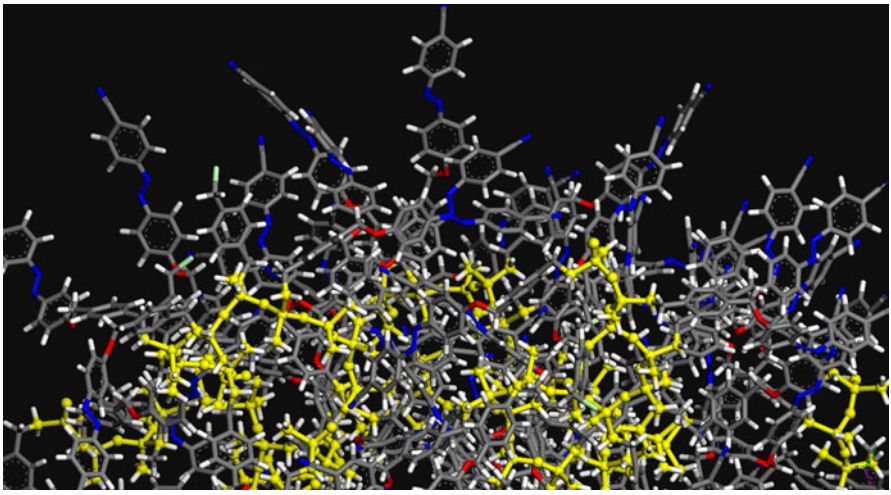

Fig. 9 Molecular simulation of a polymeric layer corresponding to Sample 7

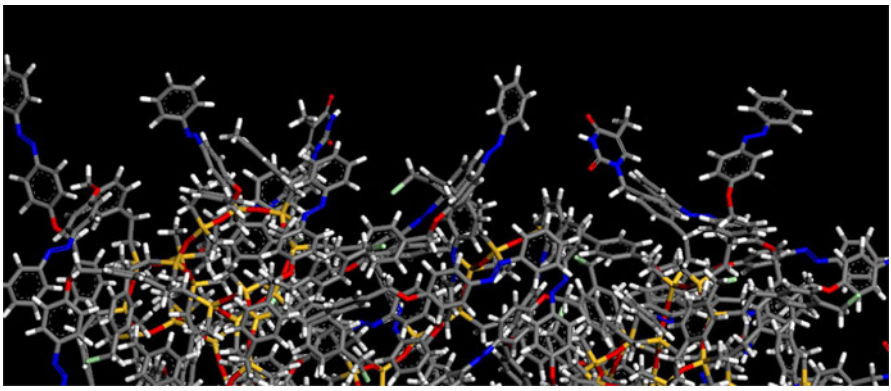

Fig. 10 Molecular simulation of a polymeric layer corresponding to Sample 8

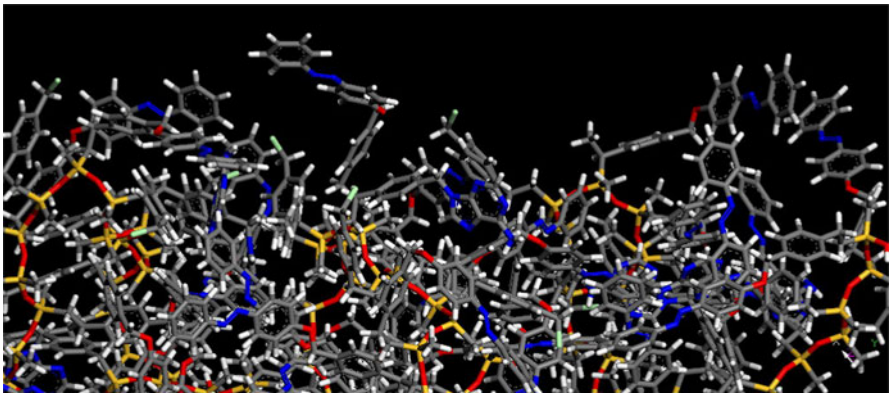

Fig. 11 Molecular simulation of a polymeric layer corresponding to Sample 9

Conclusions

In this study, we have investigated the surface properties and the photochromic behavior of some azo-polysiloxanes modified with nucleobases, materials with potential applications in biomolecules immobilization and nano-manipulation. Regarding the photochromic behavior, it could be noticed that the presence of nucleobases do not disturb the *cis*/*trans* relaxation of the azobenzene groups. Restructuring is accompanied by *cis*-azo pivoting around the main-chain and their transition towards the secondary layers. This mechanism is supported by the AFM studies, showing a reduction of the adhesion upon UV exposure. Depending on the chemical composition of the azo-polysiloxane, the values of the adhesive force can be modified. Preliminary studies of dsDNA immobilization onto the film surfaces have shown that this attachment is also a result of the chemical composition. Once immobilized, dsDNA is only slightly desorbed by varying the electrolyte composition, excepting Sample 10. The molecular modeling studies strongly

suggest that the orientation of the azo groups, relative to the film surface, could be responsible for the DNA immobilization.

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