



**HAL**  
open science

## Azobenzene based polymers as photoactive supports and micellar structures for applications in biology

Licínio Rocha, Cristina-Maria Paius, Alina Luca-Raicu, Elena Resmerita, Anca Rusu, Ioana-Andreea Moleavin, Matthieu Hamel, Norica Branza-Nichita, Nicolae Hurduc

### ► To cite this version:

Licínio Rocha, Cristina-Maria Paius, Alina Luca-Raicu, Elena Resmerita, Anca Rusu, et al.. Azobenzene based polymers as photoactive supports and micellar structures for applications in biology. *Journal of Photochemistry and Photobiology A: Chemistry*, 2014, 291, pp.16-25. 10.1016/j.jphotochem.2014.06.018 . cea-01820327

**HAL Id: cea-01820327**

**<https://cea.hal.science/cea-01820327>**

Submitted on 1 Aug 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Azobenzene based polymers as photoactive supports and micellar structures for applications in biology

Licinio Rocha <sup>a,\*</sup>, Cristina-Maria Păius <sup>b</sup>, Alina Luca-Raicu <sup>b</sup>, Elena Resmerita <sup>a,b</sup>,

Anca Rusu <sup>a</sup>, Ioana-Andreea Moleavin <sup>c</sup>, Matthieu Hamel <sup>a</sup>, Norica Branza-Nichita <sup>d</sup>, Nicolae Hurduc <sup>b</sup>

<sup>a</sup> CEA, LIST Saclay, Laboratoire Capteurs et Architectures Électroniques, 91191 Gif-sur-Yvette Cedex, France

<sup>b</sup> “Gheorghe Asachi” Technical University of Iasi, Department of Natural and Synthetic Polymers, 73, Prof. Dimitrie. Mangeron Street, 700050 Iasi, Romania

<sup>c</sup> Advanced Research Center for Bionanoconjugates and Biopolymers, Institute of Macromolecular Chemistry “Petru Poni”, 700050 Iasi, Romania

<sup>d</sup> Institute of Biochemistry of the Romanian Academy, Splaiul Independentei 296, Bucharest, Romania

## Abstract

Azo-polymers have been investigated for the large structural modifications occurring under light excitation. Photo induced isomerizations of the azobenzene molecular units can provide cooperative forces able to affect self-assembling processes in the liquid state or leading to an efficient mass transport in the solid state, which results in large deformations of film surfaces.

We introduce here our studies on azopolymers based on a polysiloxane matrix bearing specific chemical functions, whose composition can be finely tuned for applications in the biological field. Depending on their chemical structure, these materials are able to form photoactive surfaces with adjustable topographic properties or photosensitive micellar architectures in aqueous solvents. In the first case, the ability to control the surface shape at the optical wavelength scale aims to provide photoactive cell growth supports with tuneable properties, for the investigation of the environment influence on cell development. The optical properties of the materials are presented, as well as the preliminary studies showing the materials potential to modify the cell response to the surface. The stability of the films surface in contact with biological media and the implications on the cell behavior are also addressed. The second property involving formation of micellar structures is demonstrated by showing the ability of the materials to encapsulate and provide controlled release of small molecules pointing to their potential use in drug delivery applications.

## Keywords

Azo-polysiloxanes, Surface grating, Cell culture, Micelles

## 1. Introduction

Polymers that are able to experience large structural changes under external stimulation, due to modifications of their chemical and physical properties, have been the subject of intense research in the past two decades. Processed as solutions, films or bulk materials, these responsive polymers can generate original functionalities with interesting prospects for a wide range of applications. Besides the sensing field, where implementation of responsive materials appears to be a straightforward approach as demonstrated with research on biological and

chemical detection [1], biological applications represent another predilection area for those polymers. An illustration is given through the developments performed on polymers able to self assemble as 3 dimensional micelle like structures in aqueous media. In particular the ability to control the assemblies' organization and stability with temperature, pH, magnetic or light fields has attracted a strong interest for applications involving the encapsulation, transport and triggered release of active molecules. Studies on a large variety of polymeric architectures were performed in this direction as reported in different article reviews [2], [3], [4].

Responsive materials implemented as bulk or thin films provide as well original tools for biological applications. For example, developments on such materials became of particular interest in the field of tissue engineering. The ability to control the surface's chemical and physical properties of cell culture supports using specific stimulations reveals to be a versatile mean to investigate the interactions of biological cells with the extra cellular matrix (ECM). The ECM can be described as a scaffold of physical and chemical signals regulating the cells processes of differentiation, growth and organization in 3 dimensional architectures [5]. As well, cells can emit their own signals through the secretion of adhesion proteins leading to a remodeling of their environment [6], [7], [8], an interplay reflecting the complexity of the dynamical mechanisms involved in tissues construction.

To investigate the interaction of cells with the ECM, nanostructuring tools have been implemented for the accurate control they provide on the cell environment properties, from sub-cellular down to the nanometer scale. Cells adhesion, growth, migration and proliferation properties were demonstrated to be affected by nano to micrometer scale topographic modifications of their growth support depending on the surface structure characteristic parameters [9], [10], [11], [12]. For example micropatterns have been used to orient the cellular growth in specific directions tailored by the surface pattern properties [13], [14], [15]. Mainly produced through conventional optical lithographic techniques, the resulting cell support properties present however the limitation to be static. A clear advantage of responsive materials relies on the ability to modulate dynamically the support properties opening further opportunities in understanding the dynamical relationship between cells and their environment.

In particular, azoic materials appear as promising ECM to investigate the cell–environment interactions. Discovered in 1995 by two independent groups [16], [17], the efficient mass transport optically induced in azo-polymer films can be implemented to generate large and reversible topographic modifications at the optical wavelength scale in correlation with the intensity pattern. Azo-polymer films behave as a photographic plate in which variations of an image intensity can be recorded as a film thickness modulation enabling a variety of structures to be produced. A very important specificity of these photographic plates relies on the possibility to reinitialize the plate surface through additional thermal or optical treatment of the films [18], [19]. While the complete mechanism of the deformation is still debated, the observed mass transport is established to originate on molecular motions resulting from the azobenzene photo-induced isomerizations. Noteworthy, the photo-induced motion produced at the molecular scale were demonstrated to generate a force able to bend or deform amorphous or liquid crystalline polymer films exposed to light with specific polarization states [20], [21]. Widely investigated in the optical and optoelectronic areas [22], [23], [24],

[25], [26], [27], [28], the interest on such materials for cell growth applications is more recent as reported with few studies performed on azo-polymethacrylate materials [29], [30], [31].

Here we present a novel class of biomaterials incorporating azobenzene molecules. Flexible polysiloxane chains are employed as a matrix incorporating the azobenzene units covalently bound in the side chain. An originality of the materials proposed relies herein on the possibility to finely tune the chemical composition adding specific chemical groups to the azo-polysiloxane chains. Besides enabling the dynamic modulation of the film surfaces through optical excitation of the azobenzene molecules, the effect of the chemical signal transmitted to the biological cells can as well be investigated depending on the chemical functions added.

First results concerning the ability of the developed azopolysiloxanes to support cell adhesion and growth are presented and influence of the chemical structures and topography is evidenced. Depending on the chemical groups added to the azo-polysiloxane chains, materials able to self-assemble as micellar structures in solution can also be obtained. Besides providing materials suitable for drug delivery applications, nanostructured films can also be produced from related solutions.

## 2. AZO-polymer films optical properties

Optical induction of surface deformations has been demonstrated in a large variety of azo-materials, ranging from single crystals to amorphous molecular and polymer films [32], [33], [34], [35]. The reported deformations are based on the motions of azobenzene molecular units distributed inside the material matrix activated under illumination. The azobenzene molecules can be excited from their *trans* form to their *cis* form following absorption of a photon and can relax back to the fundamental *trans* state either optically or thermally as depicted in Fig. 1. Cooperative *trans*–*cis*–*trans* isomerizations can be efficiently converted to polymer chains conformational changes by covalent bounding of the azobenzene molecules to the polymer backbone producing then a force able to drive an efficient mass transport in the films.

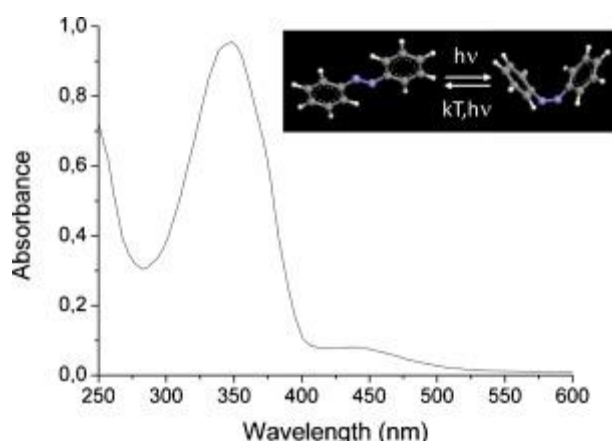


Fig. 1. Azobenzene isomerization and absorption spectrum.

Several attempts to model the conversion process of the molecular photo-induced isomerization into the observed macroscopic changes have not led yet to a comprehensive understanding of the processes involved [36], [37], [38], [39], [40], [41], [42], [43], [44]. In particular the mechanism enabling a mass transport to occur well below the transition temperature of the materials studied is still debated as demonstrated by contradictory experimental results [45], [46]. Studies were performed under different optical configurations

[47], [48], [49], [50] and with materials showing diverse architectures [18], [51], [52], [53], [54], [55] evidencing a strong relationship between the topographic changes and the materials structural properties.

### 3. Characterization of the azo-materials as photoactive supports

The samples investigated are based on polysiloxanic chains grafted with azobenzene molecules in the side chain and incorporating additional chemical units as depicted in Fig. 2. The synthesis procedure has been previously detailed (see for example [56]).

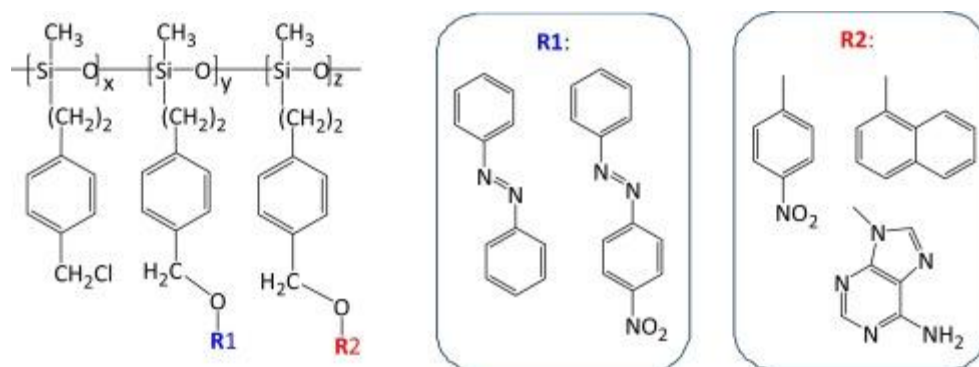


Fig. 2. Chemical structure of azo-polysiloxanes.

Mass transport properties have been widely investigated using an interference pattern characterized by a sinusoidal modulation of its intensity following an axis. As a result, a sinusoidal modulation of the film thickness is obtained revealing a mass transport correlated to the intensity pattern.

Fig. 3 schematizes the experimental set-up employed for the studies reported in the present article. A frequency doubled laser diode delivering a continuous 488 nm wavelength beam is used to illuminate the films in the azobenzene absorption band. The laser beam is divided with a beam splitter and the two resulting beams are superposed onto the film surface after reflexion onto symmetrically oriented mirrors with respect to the normal of the film surface. The result is a sinusoidally modulated interference pattern with a modulation axis given by the intersection of the beams incident plane and the film surface. The typical image obtained with an atomic force microscope (AFM) of a polymer film after illumination is also given.

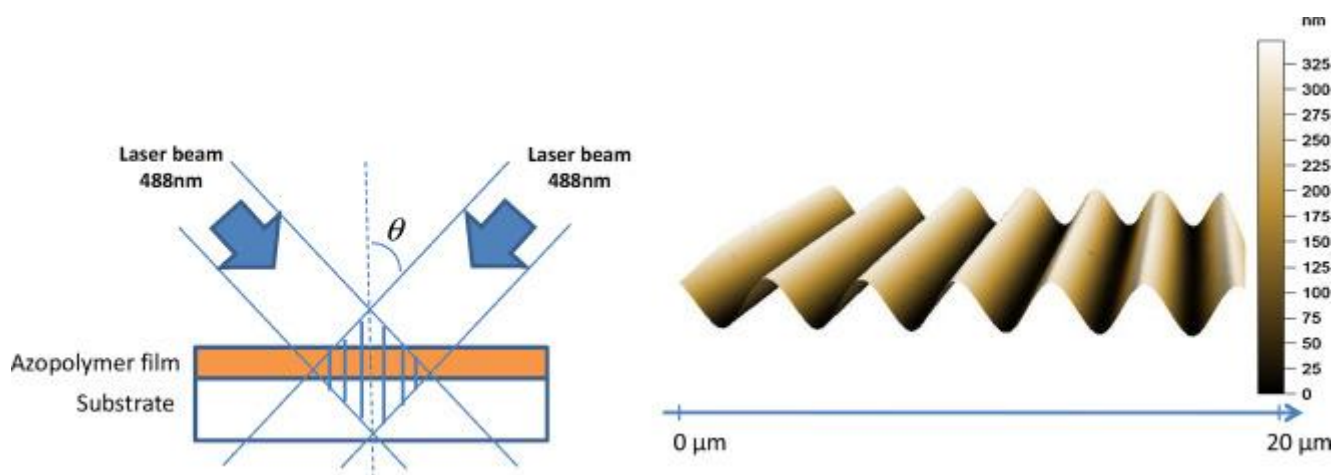


Fig. 3. Optical configuration of the azopolymer film illumination and typical induced surface relief pattern.

Besides enabling the generation of optical gratings with direct applications in optoelectronic devices, this configuration appears also to give valuable information on the mass transport process. While the period corresponds to the fringes spacing of the interference pattern and is controlled by the incident angle  $\theta$  (see Fig. 3), the modulation amplitude can be related to the mass transport efficiency.

The modulation dynamics is controlled for a given intensity exposure by the illumination time, *i.e.* the total energy absorbed by the film, and its efficiency depends also on the laser polarization state [57], [58] excluding purely thermal effects to be the cause of the induced deformations. The curves of Fig. 4 are obtained by monitoring the evolution of a 632 nm Helium–Neon 1st order diffraction intensity during the polymer illumination and represents the formation of the surface grating in time. The experiment was performed on a 400 nm thick film of a polymer (azo90-Psi) represented in the figure incorporating the azobenzene molecule with a 90% substitution degree. The mean illumination intensity was  $180 \text{ mW cm}^{-2}$  and the polarization was set perpendicular to the interference pattern fringes (*p*-polarization) to favor a mass transport from the high intensity to the low intensity areas. Also represented on the graph the result obtained with a beam polarization along the interference pattern fringes (*s*-polarization), evidencing a lower modulation efficiency.

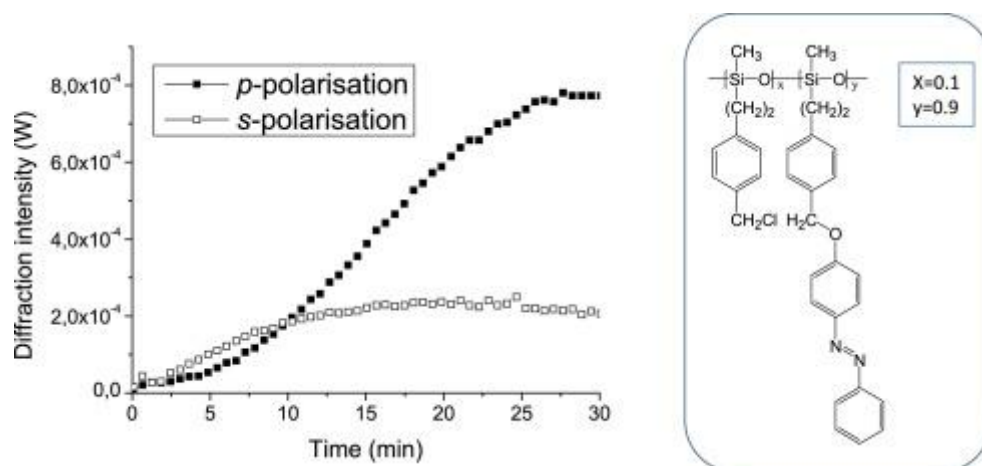


Fig. 4. Time evolution of the 1st order diffraction efficiency and chemical structure of the azo90-Psi polymer.

Incorporation of additional chemical functions to the azo-polysiloxane backbone can lead to strong modifications of physical properties and thus of the mass transport efficiency. A particularity of the polymers investigated here relies on their low glass transition temperatures ( $T_g$ ) which can be modulated between less than  $20 \text{ }^\circ\text{C}$  up to  $70 \text{ }^\circ\text{C}$  depending on the chemical groups and the substitution degree on the polysiloxane chains. The polymer film  $T_g$  appears as a critical parameter in particular when it is close to the operating temperature, the film viscosity impacting the deformation mechanism and surface stability [54], [59], [60], [61], [62]. As well, a difference in the chemical structure of materials with similar  $T_g$  can reveal remarkable and unexpected properties when the operating temperature is approaching the  $T_g$ . An example is given in Fig. 5 where the behavior of 2 materials, azo46-NO46-PSi and azo50-Naph20-PSi, exhibiting only a slight difference in their  $T_g$  is evidenced. The figure represents the modulation dynamics of the surface for both materials under illumination as well as the

stability of the induced deformation with time when the illumination is stopped. Along with the diffraction curves, the topography evolution of the azo46-NO46-PSi film after the laser is stopped is shown in the AFM image evidencing the relaxation of the surface modulation.

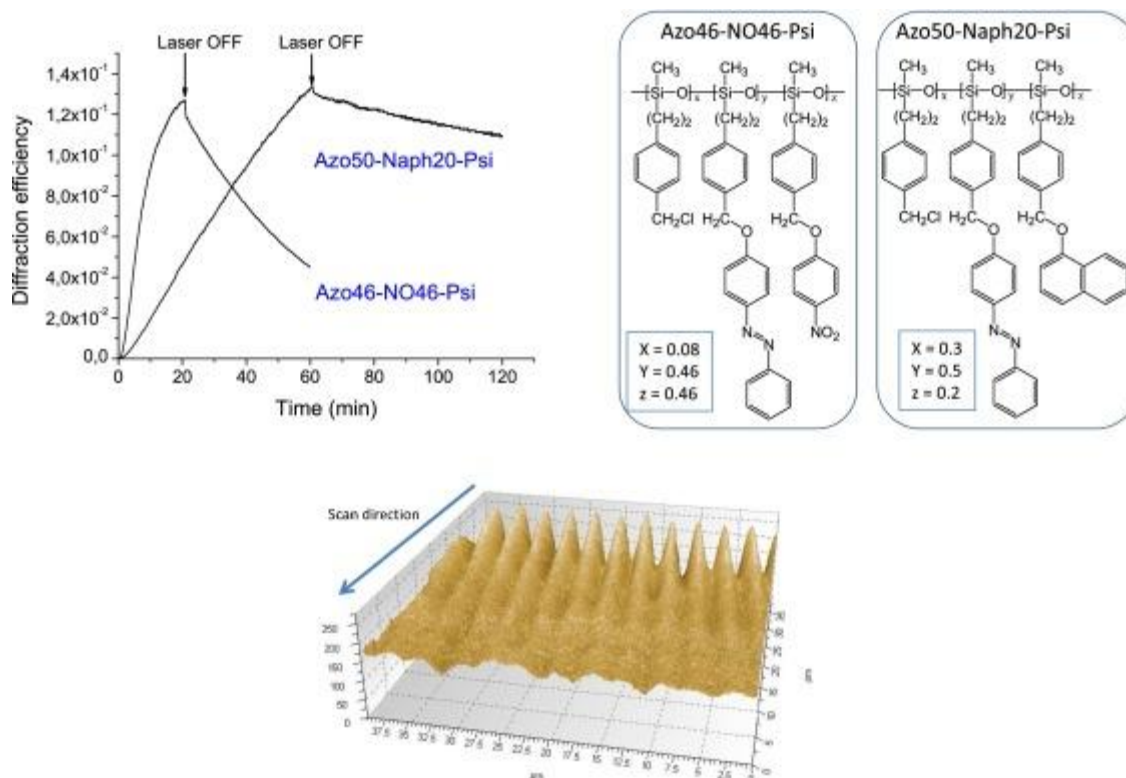


Fig. 5. azo46-NO46-PSi and azo50-Naph20-PSi polymer films modulation dynamics and stability. AFM image showing the evolution in time (*i.e.* scan direction) of a modulated azo46-NO46-PSi film surface.

The materials reported herein present equivalent substitution degrees on the polysiloxane chains as concerns the azobenzene molecules, but incorporate different additional chemical units, nitrophenol for azo46-NO46-PSi and Naphthol for azo50-Naph20-PSi, with 46% and 20% substitution degree respectively. The resulting  $T_g$  are respectively 27 °C and 25 °C. The experiments were performed with an operating temperature of 25 °C corresponding to the  $T_g$  of azo50-Naph20-PSi.

While the  $T_g$  is slightly higher for azo46-NO46-PSi, the modulation process is faster and the stability of the induced modulations at the film surface appears weaker than for azo50-Naph20-PSi. The observed phenomena cannot be explained only by differences in the polymer chains flexibilities and the competition occurring between the photoinduced directed mass transport and the random thermal redistribution of the polymer chains within the film. Besides a modification of the polymer chains flexibility, physical inter- or intra-chains interactions provided by the additional chemical units on the azo-polysiloxane chains have to be taken into account to explain the higher stability of the photo-induced topographic modifications for the lowest  $T_g$  material [59].

Stable surfaces can be obtained in those polymers by increasing the substitution degree on the polysiloxane chains. For example an increase of the Naphthol substitution ratio to 27% increases the material's  $T_g$  to 40 °C and as a result leads to a strong decrease of the modulation dynamics but produces stable surface gratings with time [59].

The possibility to modulate the stability of the induced topographic modifications enables the production of surfaces whose properties can be modified dynamically during the cell growth process, providing a seducing tool for cell culture applications.

#### 4. Substrates for cell growth applications

Preliminary studies were performed to evaluate the compatibility with cellular adhesion and proliferation of the synthesized azo-polysiloxanes incorporating different azo-molecules as well as additional chemical functions.

The studies were performed on a human hepatoma-derived cell line, HepaRG, using confocal microscopy and fluorescent markers to reveal the structural details of the cells, as a direct indication of their attachment and adherence to the substrates [63].

Fig. 6 shows the typical response of hepatic cells to 3 different azo-polysiloxane films compared with a standard tissue culture coverglass as a control surface. The nuclei appear in blue and the distribution of the actin and tubulin cytoskeleton elements is shown in green and red respectively. The first film, azo90-Psi, is a polysiloxane substituted by the 4-phenylazophenol molecule with an 85 ratio. The second one, azo63-Ad18-Psi, incorporates the same azo-molecule at a smaller ratio enabling the incorporation of adenine nucleobase as additional chemical units with a 18% substitution degree. The last material, azoNO<sub>2</sub>83-Psi, is a polysiloxane modified by a more polar azo-molecule, the 4-((4'-nitrophenyl) azo)phenol, with a similar substitution degree as the first material. The film thickness is close to 1 μm for azo90-Psi and azoNO<sub>2</sub>83-Psi, and 700 nm for azo63-Ad18-Psi. The chemical structures and substitution degrees of the materials are shown in Table 1.

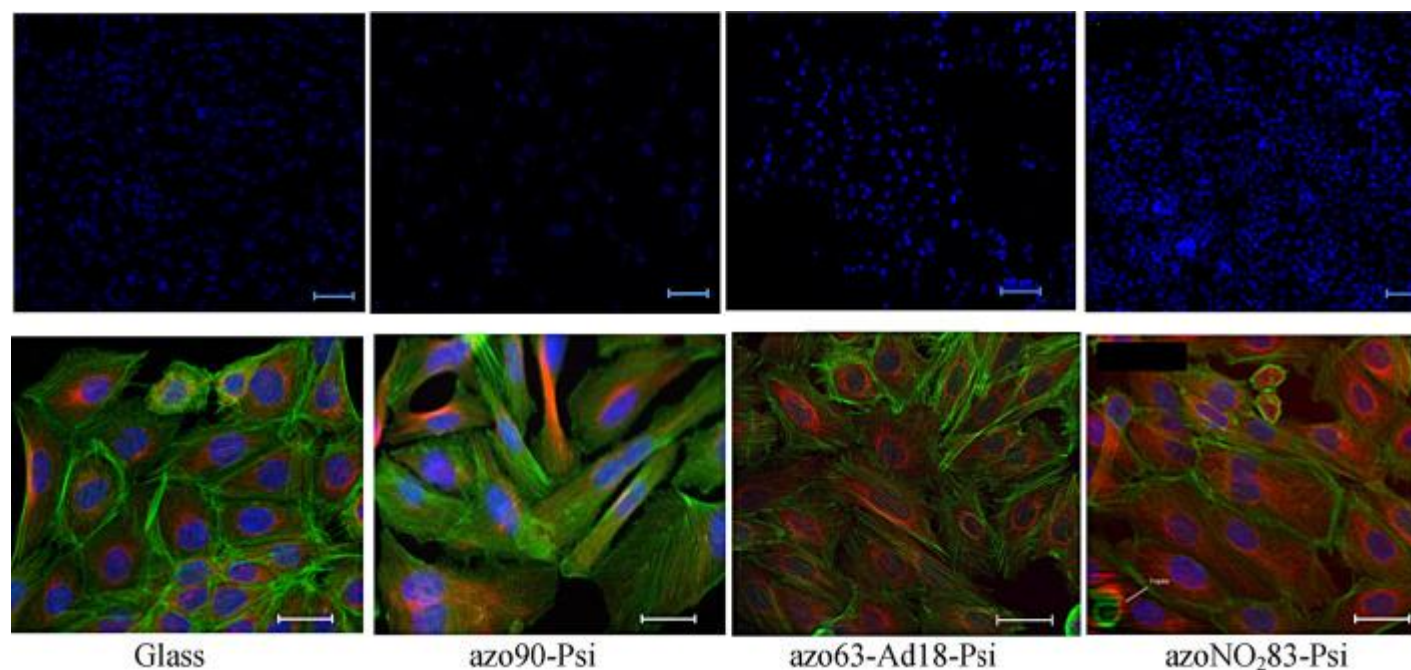


Fig. 6. Immunofluorescence microscopy images of cells grown on a standard control substrate and on azo-polymer films with the different chemical structures introduced in Table 1. The cell nuclei are stained in blue and the cytoskeleton element organization is shown in green and red representing the actin filaments and the microtubules. The scale bar is 100 μm (top line) and 50 μm (middle line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



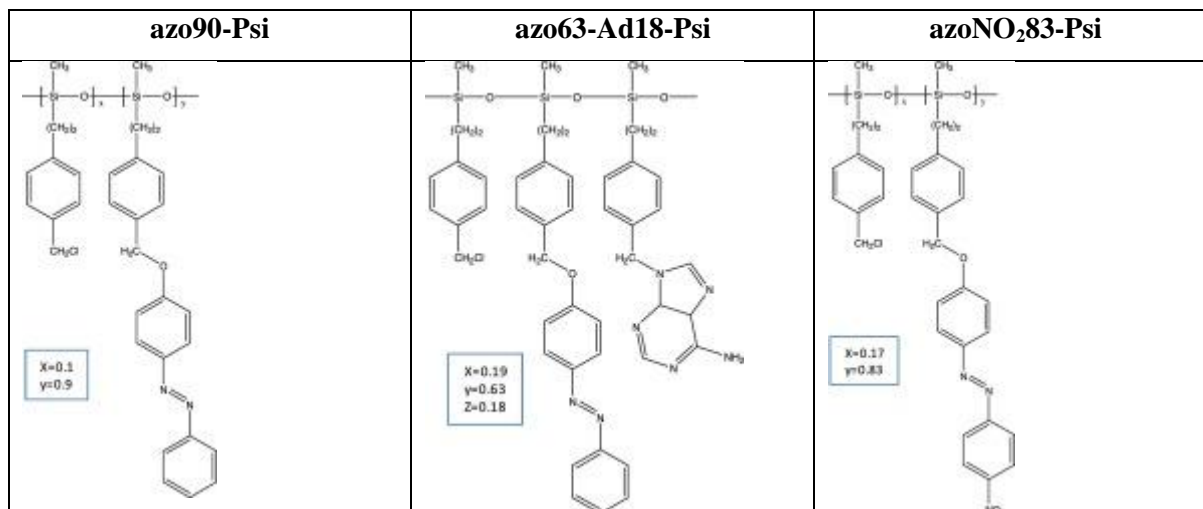


Table 1. Chemical structure of the polymers presented for the cell culture experiments.

The figure evidences clearly the influence of the polymer support onto the cell culture behaviors. Compared to the control substrate and other polymer films, the azo90-Psi film demonstrates poorer cell adhesion properties as revealed by disorganized actin filaments and microtubules, and a low number of cells attached to the surface in 24 h after seeding. The incorporation of adenine nucleobase onto the azo-polysiloxane chains with the sample azo63-Ad18-Psi appears to improve the affinity of the polymer for the cells as evidenced by the increased number of cells attached onto the film surface. However, the cell adhesion properties of this polymer are still weaker than those of the control surface showing a higher number of attached cells. The polymer with the best properties for cell development is the azoNO<sub>2</sub>83-Psi. This material promoted adhesion of a larger number of cells compared to the other samples; moreover it appeared to induce formation of cell clusters, as judged from the high cellular density and the disposal of the actin filaments toward the cell plasma membrane. This process, which is highly relevant for the HepaRG differentiation [63] will be investigated in molecular detail in the near future.

The cells grow also well on the azo63-Ad18-Psi and azoNO<sub>2</sub>83-Psi films, taking into consideration the appearance and distribution of the actin filaments and microtubules resembling those of the cells grown on the control surface.

While the chemical structure and the rheological properties of the films have a direct effect on the cell behavior, the polymer surface re-organization during exposure to the aqueous culture medium is also a critical parameter to consider for cell culture applications.

The consequences of the film exposure to water were thus investigated further for the 3 materials presented above. The surface evolution was imaged with the AFM operated in the acoustically driven mode (tapping) in a liquid cell to enable direct measurements of the films surface while exposed to water. Fig. 7 shows the topography obtained for the polymer films with an equivalent thickness close to 1  $\mu\text{m}$  laid onto a glass substrate and exposed to water for 3 h.

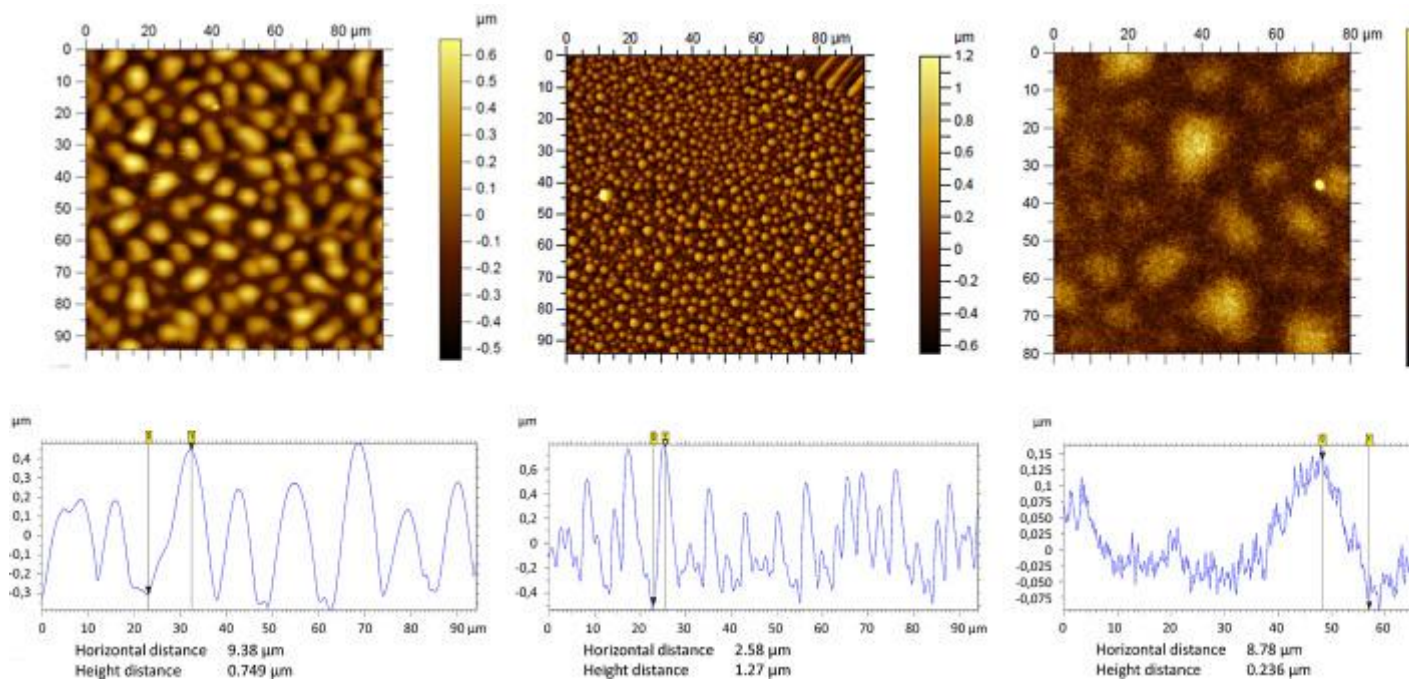


Fig. 7. AFM images of azo-polymer films surfaces in contact with water: Azo90-Psi (left), Azo63-Ad18-Psi (middle) and AzoNO<sub>2</sub>83-Psi (right). The films were realized onto a glass substrate.

A very different evolution of the films, showing initially flat surfaces with roughness ranging from 0.6 nm for the Azo63-Ad18-Psi film to 33 nm for the AzoNO<sub>2</sub>83-Psi film, is observed after exposure to water. Following 3 h of contact with water the films display an “island” like surface with different shapes. While the azoNO<sub>2</sub>83-Psi exhibits a relatively good stability of its surface with modulations reaching 300 nm in height for 10–30 μm width, the induced structures for two other films present higher spatial frequencies. In the case of azo90-Psi the amplitudes of the islands can reach more than 800 nm for lateral sizes around 10 μm, and for the azo63-Ad18-Psi amplitudes of 1 μm for lateral sizes around 3 μm can be obtained. The films were obtained using the same experimental conditions, which suggests a direct connection of the observed modifications with the chemical structure of the materials. Depending on the material polarity and rigidity, the films may re-organize differently their structure in response to the contact with water. In the case shown here, the more stable film is the one incorporating the azo-molecules with the higher polarity and rigidity, with a  $T_g$  of 48 °C as compared, for example, to 36 °C  $T_g$  of the azo90-Psi.

A first conclusion can be drawn from these observations when correlated to cell behavior described above. While the relatively flat surface of the azoNO<sub>2</sub>83-Psi film promotes cell adhesion and proliferation, the higher spatial modulation frequencies shown for azo63-Ad18-Psi appear also to favor the cell development, as compared to the intermediate spatial surface modifications undergone by the azo90-Psi film. This confirms the importance of the polymer physical and chemical structure on the cell growth and explains the differences observed between various polymer samples.

To dissociate the effect of chemical and rheological signals sent to cells from the film topography influence, surfaces presenting a good stability in the presence of water have to be produced. The stability of polymer films in aqueous media is a critical parameter requiring the engineering of specific substrates, a main problem being the exfoliation of the films following

water infiltration between the polymer and the substrate surface. A solution employed by Barillé et al. consists in increasing the substrate hydrophobicity through silanization of its surface [31]. Another strategy employed here relies on the use of polymethyl methacrylate (PMMA) as substrates for the films presenting a higher hydrophobicity than the glass substrates employed.

The influence of water on the surface properties of an azo90-Psi film realized onto a PMMA substrate is represented in Fig. 8.

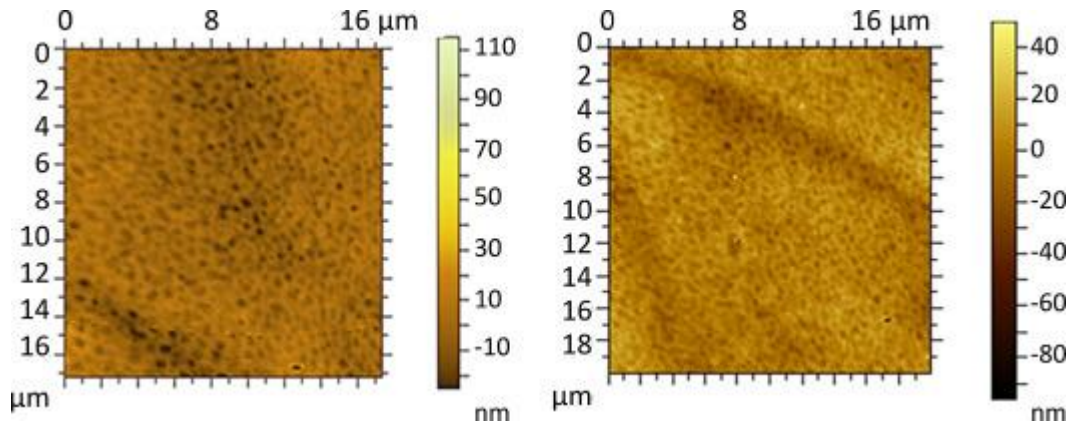


Fig. 8. AFM image of an azo90-Psi film surface before (left) and after 20 h exposition to water (right).

The film was realized onto a PMMA substrate.

The films have similar roughness of 9.2 nm before water exposure and 8.3 nm following 20 h exposure to water. The thin structures visible on the pictures correspond to the substrate topography which is transferred to the film surface. The use of this hydrophobic PMMA substrate stabilizes the surface. In the present case we expect also the solubilization of a superficial layer of the substrate to occur during the film coating (realized by spin coating from a trichloro-1,1,2-ethane solution) resulting in an improved adhesion of the polymer film to the substrate.

The influence on the cells development of the substrate employed to layer the films is observed in Fig. 9, showing the azo90-Psi and azo63-Ad18-Psi films with thicknesses between 700 and 850 nm.

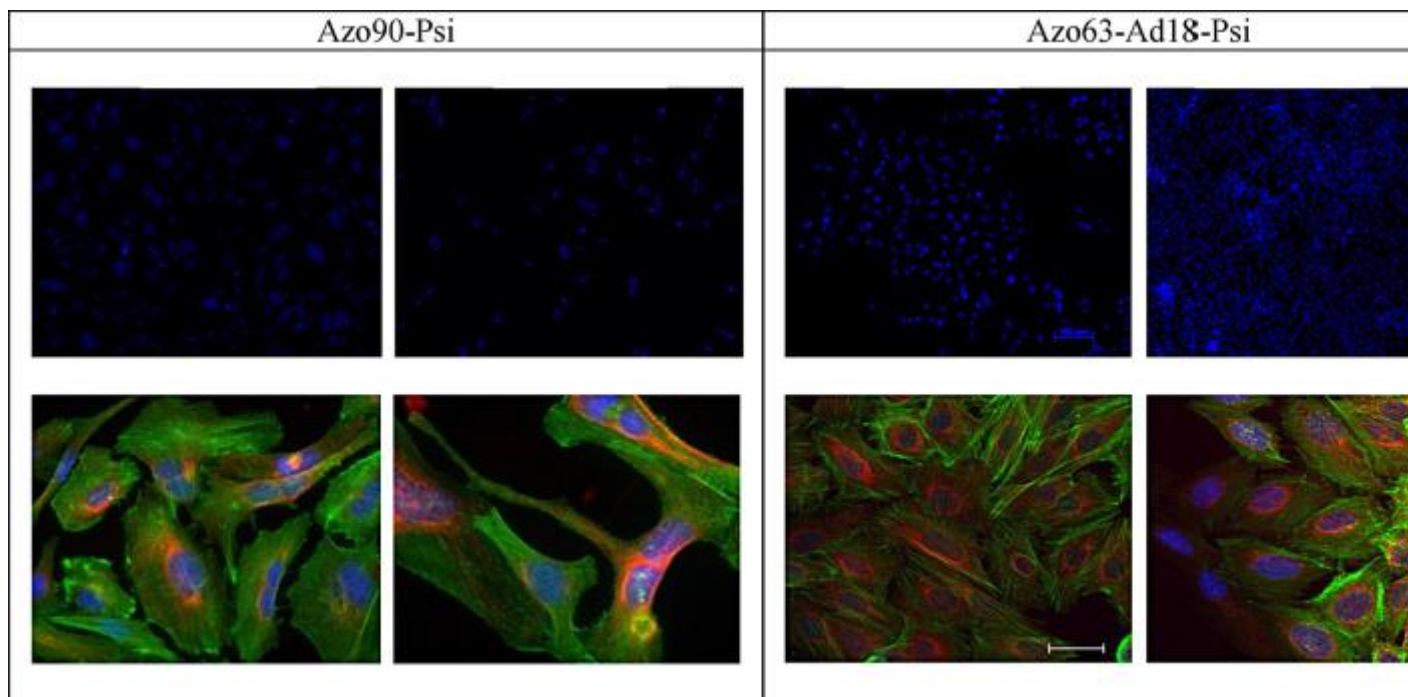


Fig. 9. Immunofluorescence microscopy images of cells grown on azo90-Psi and azo63-Ad18-Psi films. Each film was laid on either glass (left) or PMMA (right) substrates. The cell nuclei appear in blue and the cytoskeleton elements in green for actin and red for tubulin. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

For the azo90-Psi film a more stable surface appears to result in a lower compatibility of the film with the cells, since a reduced number of cells were evidenced on the film laid on PMMA. This indicates that the modulation of the film surface may improve the cell adhesion properties of this polymer. An opposite behavior was observed for the azo63-Ad18-Psi film, an increased cell adhesion and proliferation rate being obtained for the film on PMMA.

This experiment clearly shows the possibility to influence the cell culture development by modifying the film topography of a material with a certain chemical structure. The next step will be the investigation of controlled topographic modifications induced optically with the aim to demonstrate the possibility to influence the cell development. In this respect the spontaneous modulation undergone by the films when exposed to water gives a first indication of surface modulation parameters favoring the cell adhesion and proliferation.

The possibility to modulate dynamically the surface properties with an optical excitation along with the chemical and physical properties through chemical engineering, is an original feature of this class of biomaterials, opening new opportunities for the investigation of the dynamics of the cell–environment interactions.

An important parameter to be taken into account in further experiments concerns the effect of the film thickness. For the films investigated here with thicknesses ranging from a few hundreds of nanometers to the micrometer range, the mechanical properties, *i.e.* the film elasticity and viscosity, are strongly influenced by the substrate and are different from the bulk properties. We have recently evidenced important modifications of the elasticity modulus magnitude of azo-polysiloxane films with different chemical compositions as a function of the film thickness. While the films exhibit an elastic behavior with a modulus in

the MPa domain for thickness of several hundreds of micrometers, they are acting like rigid bodies with an elasticity modulus reaching the GPa domain when the thickness is reduced to several hundreds of nanometers [64].

Besides showing different surface behaviors when exposed to water, polymer films with different thickness are thus expected to influence the cellular adhesion and migration differently. As well, the effect of the materials elasticity can be investigated through the synthesis of materials presenting different  $T_g$  above or below the 37 °C operating temperature. Further experiments are currently under way.

## 5. Micellar structures

Using specific hydrophilic groups as additional chemical function incorporated onto the azo-polysiloxanes, materials which are able to self-assemble as micellar structures in aqueous environments can be produced. The aggregation capacity of amphiphilic azo-polysiloxanes have been evidenced in previous studies [65]. Compared to block copolymers, in the case shown here, the hydrophilic additional chemical functions and the hydrophobic azobenzene molecules are statistically distributed along the polysiloxane chains. A possible explanation of the micellar aggregation capacity can be provided by the flexibility of the polysiloxane main-chain enabling the arrangement of the hydrophilic and the hydrophobic groups on both sides of the polymeric chain.

The incorporation of azo-molecules within such polymeric architectures can provide an optical triggering mechanism to control the assemblies aggregation/disaggregation process, through the isomerization induced dipolar changes of the molecules [2], [66], [67]. Combined with their loading capacity of small molecular units, these optically responsive polymeric systems offer promising perspectives for drug delivery applications.

An illustration is given in this section with a copolymer (IM24) based on a polysiloxane incorporating in the side chain the photo-active 4-phenylazophenol molecule and a triethyl amine as the hydrophilic group with a 30% and 7% substitution degree respectively. The chemical structure of the polymer is depicted in Fig. 10. The synthesis procedure of similar polymers using different tertiary amines was detailed previously [66], [68].

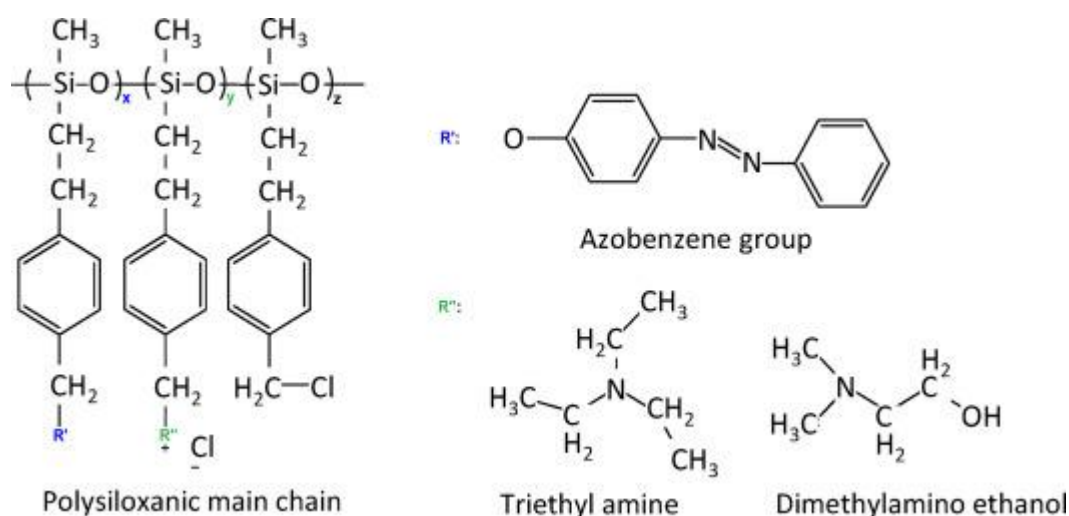


Fig. 10. Chemical structure of an amphiphilic azo-polymer (IM24).

The amphiphilic azo-polymers aggregation capacity can be evaluated with the classical method using pyrene fluorescence spectroscopy [69]. The critical concentration of

aggregation (*CCA*) value is calculated monitoring the evolution of the first ( $I_1$ ) and the third ( $I_3$ ) vibrational peaks intensity corresponding to the fluorescence emission spectrum of pyrene, which ratio depends on the environment polarity (Fig. 11) [70].

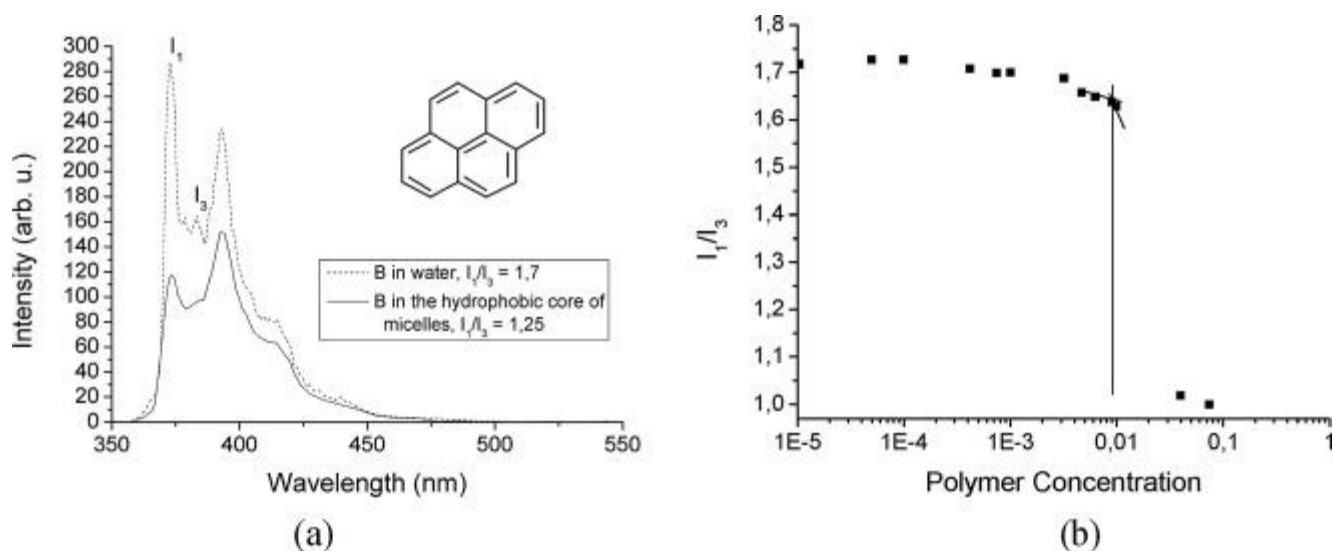


Fig. 11. (a) Fluorescence emission spectra for pyrene in water and in the hydrophobic core of micelles. The chemical structure of the pyrene is also given. (b) Plot of the  $I_1/I_3$  ratio evolution as a function of polymer concentration in water for sample IM 24.

In aqueous solution, the  $I_1/I_3$  ratio value corresponding to the free pyrene (not incorporated in the micelles) is located in the range 1.70–1.75. The evolution of the  $I_1/I_3$  ratio with the polymer concentration indicates aggregation starts for concentrations higher than  $10^{-3} \text{ g L}^{-1}$ : the value of the  $I_1/I_3$  ratio around 1.7 below this concentration is progressively decreasing for increasing concentrations reflecting the progressive encapsulation of the pyrene molecules into the hydrophobic core of the forming aggregates.

The *CCA* value is estimated as the first inflexion point of the curves. The *CCA* is situated in a low concentration domain between  $10^{-2}$  and  $10^{-3} \text{ g L}^{-1}$  what can be explained by the presence in the polymer side-chain of azobenzenic hydrophobic groups, with a high aggregation tendency [71].

The presence of micellar structures is also confirmed by Dynamic Light Scattering (DLS) and Scanning Electron Microscopy (SEM) as shown in Fig. 12.

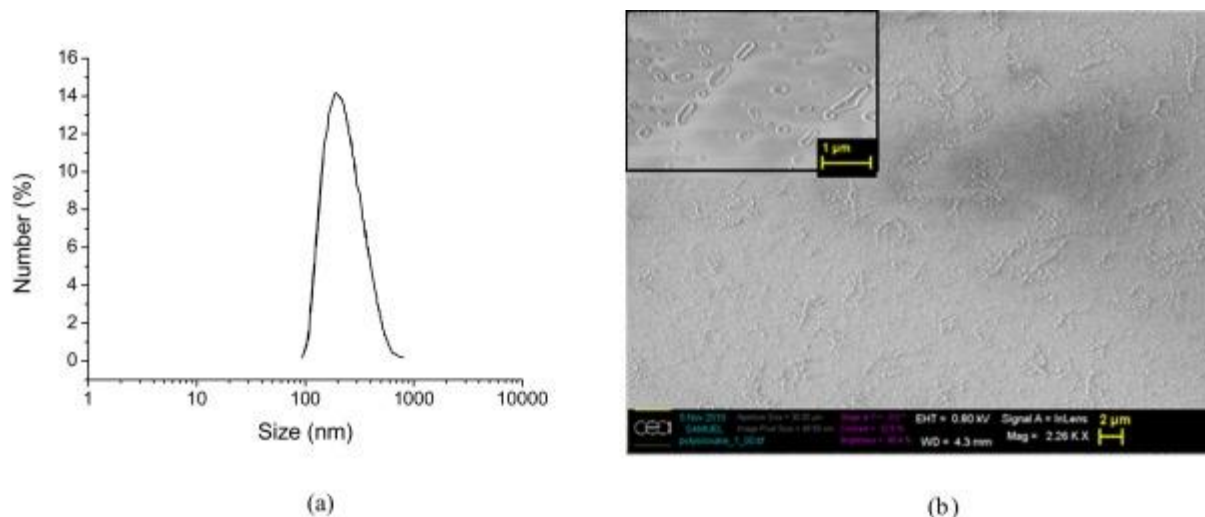


Fig. 12. (a) DLS curves for a  $0.25 \text{ g L}^{-1}$  solution in water of sample IM 24 and (b) SEM image obtained after evaporation of a droplet from a  $0.25 \text{ g L}^{-1}$  solution of same sample.

For a  $0.25 \text{ mg mL}^{-1}$  polymer concentration, the solution is characterized by a large population with aggregates sizes centered around 200 nm. The high polydispersity of the solution is also confirmed by the SEM images showing the presence of both large and small size aggregates exhibiting circular or more elongated shapes.

The polymeric micelles ability to encapsulate and release small molecules when an optical excitation is applied to the azo-molecules can be monitored in real time, using fluorescent molecules whose fluorescence intensity is quenched in polar environments. In our experiment, fluorescent Nile Red was incorporated in the polymeric aggregates and continuously excited at 550 nm wavelength. The red shifted emission of the molecule centered around 600 nm enables indeed to use an excitation wavelength outside the absorption range of the azo-molecules.

Fig. 13 represents the variation of the Nile Red emission at 640 nm under continuous excitation at 550 nm, as a function of time, in a water solution containing the polymer at  $0.015 \text{ mg mL}^{-1}$ . The evolution of the Nile Red emission was investigated using additional illumination of the azobenzene molecule in the UV range (full symbols). The evolution of the Nile Red emission under the same excitation conditions in a non photo-active test sample made of a polysiloxane incorporating only dimethylamino ethanol units with 17% substitution degree as the hydrophilic group is also shown (empty symbols).

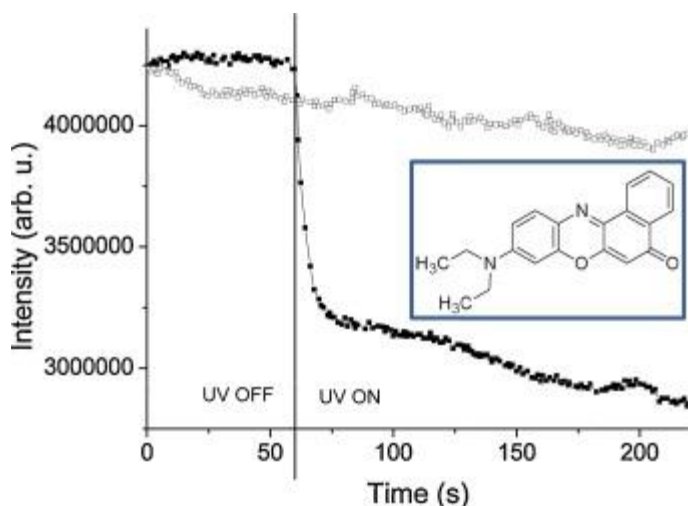


Fig. 13. Variation as a function of time of the fluorescence intensity at 640 nm, for micelles with encapsulated Nile Red in water. The solution is exposed to a 550 nm continuous illumination (excitation of the Nile Red). UV irradiation (excitation of the azo-molecules) is added after 50 s. The Nile Red chemical structure is also given.

For the sample with photo-sensitive micelles, a stable signal for the first 60 s is observed until the exposure to UV light leading to a sharp decrease of the Nile Red fluorescence intensity followed by a slower decrease. This intensity decrease is attributed to the modification of the Nile Red environment polarity resulting from a release of the Nile Red molecules in the water following disruption of the micelles. An increase of the micelles core polarity induced by azo-molecules isomerizations without a destruction of the micelles may as well contribute to the observed decrease of intensity.

We also observe that the UV illumination of the non-sensitive micelles does not result in a modification of the Nile Red fluorescence intensity confirming that the observed decrease of the fluorescence intensity for the photo-sensitive sample is not due to a degradation of the fluorescent molecule under the UV irradiation.

The excitation with UV produces additional fluorescence of the Nile Red molecule appearing as an offset which has been suppressed in the graphs.

The chemical structure of the materials will have a strong influence on the organizational properties of the polymer chains in solution, in particular through the ratio between the hydrophobic azobenzene and the hydrophilic amine and the chain polymer length. Tuning of the hydrophilic/hydrophobic balance by modifying the content ratio of the azobenzene and amine molecules can impact the size, the CCA and the stability of the nanostructures in solution. Chemical engineering provides thus an efficient mean to optimize the properties of the materials for drug delivery applications. For example, increasing the amine content led to an improved stability of the micelles but with a consequent reduction of their diameter down to 20 nm with an expected impact on the loading capacity. As well, the disruption dynamics and efficiency under optical excitation will be affected by the chemical structure. More detailed results will be reported in a further publication.



## 6. Conclusion

We have shown here the potential of azo-polysiloxanes for biological applications in at least two main directions. Depending on their chemical structure, the polymers can be used as thin films or can self-assemble as nanoparticles when dispersed in aqueous solutions. In the first case, the polymers can provide optically active supports to facilitate our understanding of the complex mechanisms involved in the interaction between cells and their environment. In the second case the aim is to produce nanoparticles able to encapsulate active substances with a perspective for drug delivery applications.

A specificity of azo-polymers is the real time control of the films topographic properties provided by the underlying photo-induced mass transport mechanism. Reversible nano and micro-patterns can be produced on the surface of azo-polymer films through a single step exposure to an interference pattern enabling a dynamic control on the film surface. The originality of the studies presented here relies on the fine control over the materials properties, which can be obtained through the incorporation of azo-molecules and additional chemical functions with controlled substitution degrees. Besides tuning the films opto-mechanical properties and surface modifications stability, films with different chemical compositions can be used in cell cultures. In this respect we demonstrated a good compatibility of our materials with biological cells. The chemical structure and the film topography have shown a strong influence on the cell morphology and development. In this respect the stability of the polymers surface when exposed to water is also a key parameter for the cell adhesion and proliferation and can be controlled by the chemical composition of the material. Stabilization of the film surface under aqueous media can be improved by adapting the film substrate.

Materials self-assembling in aqueous solvents as nanoparticles, which are able to encapsulate molecules have also been introduced. The possibility to optically destabilize the polymer chains 3-dimensional organization resulting in the triggered release of the encapsulated molecules in solution has been demonstrated. Optimization of the particles properties, *i.e.* stability, loading capacity and materials aggregation/disruption dynamical properties will require further investigations to correlate the materials chemical structure and organizational properties.

## References

- [1] J. Hu, S. Liu. Responsive polymers for detection and sensing applications: current status and future developments. *Macromolecules*, 43 (2010), pp. 8315-8330
- [2] C.J.F. Rijcken, *et al.* Triggered destabilisation of polymeric micelles and vesicles by changing polymers polarity: an attractive tool for drug delivery. *J. Cont. Rel.*, 120 (2007), pp. 131-148
- [3] T.O. Kyung, *et al.* Polymeric nanovehicles for anticancer drugs with triggering release mechanisms. *J. Mater. Chem.*, 17 (2007), pp. 3987-4001
- [4] F. Meng, Z. Zhong, J. Feijen. Stimuli-responsive polymersomes for programmed drug delivery. *Biomacromolecules*, 10 (2009), pp. 197-209
- [5] L.G. Griffith. Polymeric biomaterials. *Acta Mater.*, 48 (2000), pp. 263-277

- [6] E. Ostuni, L. Yan, G.M. Whitesides. The interaction of proteins and cells with self-assembled monolayers of alkanethiolates on gold and silver. *Colloids Surf. B: Biointerfaces*, 15 (1999), pp. 3-30
- [7] F. Rosso, *et al.* From cell-ECM interactions to tissue engineering. *J. Cell. Physiol.*, 199 (2004), pp. 174-180
- [8] M. Larsen, *et al.* The matrix reorganized: extracellular matrix remodeling and integrin signaling. *Curr. Opin. Cell Biol.*, 18 (2006), pp. 463-471
- [9] Y.W. Fan, *et al.* Culture of neural cells on silicon wafers with nano-scale surface topograph. *J. Neurosci. Methods*, 120 (2002), pp. 17-23
- [10] C.-H. Choi, *et al.* Cell interaction with three-dimensional sharp-tip nanotopography *Biomaterials*, 28 (2007), pp. 1672-1679
- [11] P.P. Girard, *et al.* Cellular chemomechanics at interfaces: sensing, integration and response. *Soft Matter*, 3 (2007), pp. 307-326
- [12] T. Tzvetkova-Chevolleau, *et al.* The motility of normal and cancer cells in response to the combined influence of the substrate rigidity and anisotropic microstructure. *Biomaterials*, 29 (2008), pp. 1541-1551
- [13] A.M. Rajnicek, S. Britland, C.D. McCaig. Contact guidance of CNS neurites on grooved quartz: influence of groove dimensions, neuronal age and cell type. *J. Cell. Sci.*, 110 (1997), pp. 2905-2913
- [14] E.T. den Braber, *et al.* Quantitative analysis of cell proliferation and orientation on substrata with uniform parallel surface microgrooves. *Biomaterials*, 17 (1996), pp. 1093-1099
- [15] I. Nagata, A. Kawana, N. Nakatsuji. Perpendicular contact guidance of CNS neuroblasts on artificial microstructures. *Development*, 117 (1993), pp. 401-408
- [16] D.Y. Kim, *et al.* Laser induced holographic surface relief gratings on nonlinear optical polymer films. *Appl. Phys. Lett.*, 66 (1995), pp. 1166-1168
- [17] P. Rochon, E. Batalla, A.L. Natansohn. Optically induced surface gratings on azoaromatic polymer films. *Appl. Phys. Lett.*, 66 (1995), pp. 136-138
- [18] X.L. Jiang, *et al.* Unusual polarization dependent optical erasure of surface relief gratings on azobenzene polymer films. *Appl. Phys. Lett.*, 72 (1998), pp. 2502-2504
- [19] F. Lagugné-Labarthe, T. Buffeteau, C. Sourisseau. Optical erasures and unusual surface reliefs of holographic gratings inscribed on thin films of an azobenzene functionalized polymer. *Phys. Chem. Chem. Phys.*, 4 (2002), pp. 4020-4029
- [20] D. Bublitz, *et al.* Photoinduced deformation of azobenzene polyester films. *Appl. Phys. B*, 70 (2000), pp. 863-865
- [21] C.L. van Oosten, *et al.* Glassy photomechanical liquid-crystal network actuators for microscale devices. *Eur. Phys. J. E*, 23 (2007), pp. 329-336
- [22] S.J. Zilker, *et al.* Holographic data storage in amorphous polymers. *Adv. Mater.*, 10 (1998), pp. 855-859

- [23] T. Ikeda, O. Tsutsumi. Optical switching and image storage by means of azobenzene liquid crystal films. *Science*, 268 (1995), pp. 1873-1875
- [24] Y. Shi, *et al.* Large photoinduced birefringence in an optically nonlinear polyester polymer. *Appl. Phys. Lett.*, 59 (1991), pp. 2935-2937
- [25] T. Hirose, *et al.* Azobenzene liquid-crystalline polymer for optical switching of grating waveguide couplers with a flat surface. *Opt. Commun.*, 228 (2003), pp. 279-283
- [26] J. Paterson, *et al.* Optically inscribed surface relief diffraction gratings on azobenzene-containing polymers for coupling light into slab waveguides. *Appl. Phys. Lett.*, 69 (1996), pp. 3318-3320
- [27] C. Cocoyer, *et al.* Implementation of submicrometric periodic surface structures: towards improvement of organic solar cell performances. *Appl. Phys. Lett.*, 88 (133108) (2006), pp. 1-3
- [28] C. Hubert, *et al.* Emission properties of an organic light emitting diode patterned by a photoinduced autostructuring process. *Appl. Phys. Lett.*, 87 (2005), pp. 191105-191108
- [29] H. Baac, *et al.* Submicron-scale topographical control of cell growth using holographic surface relief grating. *Mater. Sci. Eng. C*, 24 (2004), p. 209
- [30] J.K. Lee, *et al.* The topographical guidance of neurons cultured on holographic photo-responsive polymer. *Proc. 26th AIC-IEEE, EMBS, San Francisco, CA* (2004), p. 4970
- [31] R. Barillé, *et al.* Photo-responsive polymer with erasable and reconfigurable micro- and nano-patterns: an in vitro study for neuron guidance. *Colloids Surf. B: Biointerfaces*, 88 (2011), pp. 63-71
- [32] P.S. Ramanujam, N.C. Holme, S. Hvilsted. Atomic force and optical near-field microscopic investigations of polarization holographic gratings in a liquid crystalline azobenzene side-chain polyester. *Appl. Phys. Lett.*, 68 (1996), pp. 1329-1331
- [33] A. Stracke, *et al.* Gain effects in optical storage: thermal induction of a surface relief grating in a smectic liquid crystal. *Adv. Mater.*, 12 (2000), pp. 282-285
- [34] N. Reinke, *et al.* Electric field assisted holographic recording of surface relief gratings in an azo-glass. *Appl. Phys. B*, 78 (2004), pp. 205-209
- [35] H. Nakano, T. Tanino, Y. Shirota. Surface relief grating formation on a single crystal of 4-(dimethylamino)azobenzene. *Appl. Phys. Lett.*, 87 (061910) (2005), pp. 1-3
- [36] J. Kumar, *et al.* Gradient force: the mechanism for surface relief grating formation in azobenzene functionalized polymers. *Appl. Phys. Lett.*, 72 (1998), pp. 2096-2098
- [37] C.J. Barrett, A.L. Natansohn, P.L. Rochon. Mechanism of optically inscribed high-efficiency diffraction gratings in azo polymer films. *J. Phys. Chem.*, 100 (1996), pp. 8836-8842
- [38] P. Lefin, C. Fiorini, J.-M. Nunzi. Anisotropy of the photo-induced translation diffusion of azobenzene dyes in polymer matrices. *Pure Appl. Opt.*, 7 (1998), pp. 71-82

- [39] T.G. Pedersen, P.M. Johansen. Mean-field theory of photoinduced molecular reorientation in azobenzene liquid crystalline side-chain polymers. *Phys. Rev. Lett.*, 79 (1997), pp. 2470-2473
- [40] D. Bublitz, B. Fleck, L. Wenke. A model for surface-relief formation in azobenzene polymers. *Appl. Phys. B*, 72 (2001), pp. 931-936
- [41] M.L. Juan, *et al.* Multiscale model for photoinduced molecular motion in azo polymers. *ACS Nano*, 3 (2009), pp. 1573-1579
- [42] V. Toshchevikov, M. Saphiannikova, D. Neher. Microscopic theory of light-induced deformation in amorphous side-chain azobenzene polymers. *J. Phys. Chem. B*, 113 (2009), pp. 5032-5045
- [43] Y.B. Gaididei, P.L. Christiansen, P.S. Ramanujam. Theory of photoinduced deformation of molecular films. *Appl. Phys. B*, 74 (2002), pp. 139-146
- [44] A. Ambrosio, P. Maddalena, L. Marrucci. Molecular model for light-driven spiral mass transport in azopolymer films. *Phys. Rev. Lett.*, 110 (146102) (2013), pp. 1-5
- [45] N. Mechau, M. Saphiannikova, D. Neher. Molecular tracer diffusion in thin azobenzene polymer layers. *Appl. Phys. Lett.*, 89 (251902) (2006), pp. 1-3
- [46] P. Karageorgiev, *et al.* From anisotropic photo-fluidity towards nanomanipulation in the optical near-field. *Nat. Mater.*, 4 (2005), pp. 699-703
- [47] A. Ambrosio, *et al.* Light-induced spiral mass transport in azo-polymer films under vortex-beam illumination. *Nat. Commun.*, 3 (2012), pp. 1-9
- [48] C. Hubert, *et al.* Spontaneous patterning of hexagonal structures in an azo-polymer using light-controlled mass transport. *Adv. Mater.*, 14 (2002), pp. 729-732
- [49] F. Lagugné-Labarthet, T. Buffeteau, C. Sourisseau. Properties of birefringence and surface relief gratings inscribed on azopolymer thin films using various interference patterns. *J. Opt. A: Pure Appl. Opt.*, 4 (2002), pp. S235-S241
- [50] F. H'dhili, *et al.* Near-Field optics: direct observation of the field enhancement below an apertureless probe using a photosensitive polymer. *Appl. Phys. Lett.*, 79 (2001), pp. 4019-4021
- [51] N.K. Viswanathan, *et al.* Surface relief structures on azo polymer films. *J. Mater. Chem.*, 9 (1999), pp. 1941-1955
- [52] F. Fabbri, *et al.* Evidence of two distinct mechanisms driving photoinduced matter motion in thin films containing azobenzene derivatives. *J. Phys. Chem. B*, 115 (2011), pp. 1363-1367
- [53] A. Jacquart, *et al.* Influence of extrinsic and intrinsic parameters onto the formation of surface relief gratings in polar azo molecular glasses. *Dyes Pigm.*, 92 (2012), pp. 790-797
- [54] H. Ando, *et al.* Photoinduced surface relief grating formation using new polymers containing the same azobenzene chromophore as a photochromic amorphous molecular material. *Mater. Chem. Phys.*, 113 (2009), pp. 376-381

- [55] A. Sobolewska, S. Bartkiewicz. Surface relief grating in azo-polymer obtained for s-s polarization configuration of the writing beams. *Appl. Phys. Lett.*, 101 (193301) (2012), pp. 1-3
- [56] N. Hurduc, *et al.* Azo-polysiloxanes as new supports for cell cultures. *Mater. Sci. Eng. C*, 33 (2013), pp. 2440-2445
- [57] X.L. Jiang, *et al.* Polarization dependent recordings of surface relief gratings in azobenzene containing polymer films. *Appl. Phys. Lett.*, 68 (1996), pp. 2618-2620
- [58] F. Lagugné-Labarthe, T. Buffeteau, C. Sourisseau. Inscription of holographic gratings using circularly polarized light: influence of the optical set-up on the birefringence and surface relief grating properties. *Appl. Phys. B*, 74 (2002), pp. 129-137
- [59] A. Raicu Luca, *et al.* Mass transport in low Tg azo-polymers: effect on the surface relief grating induction and stability of additional side chain groups able to generate physical interactions. *Appl. Surf. Sci.*, 290 (2014), pp. 172-179
- [60] L. Mazaheri, S. Ahmadi-Kandjani, J.-M. Nunzi. Influence of temperature on the relaxation kinetics of spontaneous pattern formation in an azo-polymer film. *Opt. Commun.*, 298 (2013), pp. 150-153
- [61] J. Mysliwiec, *et al.* Dynamics of photoinduced motions in azobenzene grafted polybutadienes. *Opt. Mater.*, 33 (2011), pp. 1398-1404
- [62] P.U. Veer, U. Pietsch, M. Saphiannikova. Time and temperature dependence of surface relief grating formation in polymers containing azobenzene groups with different dipole moment. *J. Appl. Phys.*, 106 (014909) (2009), pp. 1-7
- [63] C. Petrareanu, *et al.* Comparative proteomics reveals novel components at the plasma membrane of differentiated Heparg cells and different distribution in hepatocyte- and biliary-like cells. *PLOS ONE*, 8 (2013), p. e71859
- [64] N. Hurduc, *et al.* Direct observation of athermal photofluidisation in azo-polymer films. *Soft Matter*, 10 (2014), pp. 4640-4647
- [65] I.-A. Moleavin, *et al.* Amphiphilic azopolymers capable to generate photo-sensitive micelles. *Cent. Eur. J. Chem.*, 9 (2011), pp. 1117-1125
- [66] Q. Duan, *et al.* Synthesis and thermoresponsive property of end-functionalized poly(N-isopropylacrylamide) with pyrenyl group. *J. Polym. Sci. A: Polym. Chem.*, 44 (2006), pp. 1117-1124
- [67] Y. Einaga, *et al.* Photo-functional vesicles containing Prussian blue and azobenzene. *J. Am. Chem. Soc.*, 121 (1999), pp. 3745-3750
- [68] I. Moleavin, *et al.* Photosensitive micelles based on polysiloxanes containing azobenzene moieties. *Polym. Bull.*, 65 (2010), pp. 69-81
- [69] Y. Lin, P. Alexandridis. Self-assembly of an amphiphilic siloxane graft copolymer in water. *J. Phys. Chem. B*, 106 (2002), pp. 10845-10853
- [70] S. Grama, I.-A. Moleavin, A.D. Hodorog-Rusu, N. Hurduc, I. Prisacaru, C. Ibanescu. Photosensitive azo-polysiloxanes for drug delivery applications. *Materiale Plastice*, 50 (2013), pp. 60-64

[71] J.-M. Kuiper, J.B.F.N. Engberts, B. Poolman. H-aggregation of azobenzene-substituted amphiphiles in vesicular membranes. *Langmuir*, 20 (2004), pp. 1152-1160