SURFACE MODIFICATION INDUCED BY AQEOUS ENVIRONMENT OF NUCLEOBASE-MODIFIED AZO-POLYSILOXANIC FILMS

BY

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Abstract. Taking into account the use of nucleobase-modified azo-polysiloxanic films as supports for cell cultures but also for the immobilization of biomolecules application (especially DNA, RNA) which involves contact with the aqueous environment, a study of the surface properties of these compounds was carried out by evaluating the contact angle of dry and wet surface of the samples. This study was completed and correlated with AFM behavioural studies of surfaces under aqueous media. The studied samples show a dynamic surface that can be controlled, reorganization of the polymer chains taking place depending on the chemical structure.

Keywords: contact angle, nucleobases, AFM, azo-polysiloxanes, aqueous environment.

1. Introduction

In the last years polymers containing biomolecules, and particularly those with nucleobases, represent a very attractive research area because their multiple use in material science (Lutz, 2018), nanoscience and biological field applications (Pack *et al*., 2005; Spijker *et al*., 2006; Imaz *et al*.; Cheng *et al*., 2008; McHale

et al., 2012; Amo-Ochoa and Zamora, 2014; Stylianou *et al*., 2016; Hua *et al*., 2016; Yang and Xi, 2017; Hurduc *et al*., 2013; Păiuş *et al*., 2012; Rocha *et al.*, 2014; Li *et al*., 2020).

Azo-polymers because of their amazing reversible photo-isomerization capacity, due to azobenzene molecules, are capable to be used not only in storage and optical switching (Yager and Barett, 2001) but also on cell development application and nanomanipulation of biomolecules (Karageorgiev *et al*., 2005; Hurduc *et al*., 2007; Narita *et al*., 2007).

Adhesion and proliferation of different types of cells on polymeric materials suppose contact of the polymeric film surface with cell culture medium, which contains as continuous phase aqueous media. For a better understanding of how the surface responds to contact with aqueous medium / cell culture medium, it is important to know that polymeric solids have a distinct ability to change their ordering in contact with different media, in order to minimize interfacial energy and therefore free energy of the system. If the interfacial free energy between a solid and a continuous phase of a biological fluid is small, then the thermodynamic driving force for adsorption will be small. At low values of free energy to the solid-fluid interface, it is possible to become unstable and susceptible to mechanical and/or thermal disturbances. This interfacial instability can lead to undesirable consequences such as dissolution of the solid into the fluid or absorption of the fluid and its components into the solid. Chemical composition, roughness and mobility of the polymer surface are also important (Ruckenstein and Gourisankar, 1986; Voisin *et al*., 2010; Rocha *et al.*, 2014; Hurduc *et al.*, 2013; Păiuş *et al*., 2012; Narita *et al*., 2007). While chemical composition has a direct effect on cell development, the polymer surface re-organisation during exposure to cell culture medium is a critical parameter. In consequence for characterisation of nucleobase azo-polymers – water interaction we developed a study regarding the behaviour of samples under aqueous media with the help of contact angle and AFM methods.

2. Experimental

2.1. Synthesis of azo-polysiloxanes modified with nucleobases

The azo-polymers were obtained in a two-step reaction, starting from a polysiloxane containing chlorobenzyl groups (CBZ) in the side-chain. First step involves the modification of polysiloxane with 4-hydroxyazobenzene (50 – 55% substitution degree) and the second one, the unreacted chlorobenzyl groups were substituted with azotate bases. Details concerning polymer synthesis and characterization have been previously reported (Hurduc *et al*., 2007; Sedlak *et al.*, 2003).

The synthesis reaction scheme is given in Fig. 1. The polymers chemical structure was confirmed by ^{1}H-NMR spectroscopy.
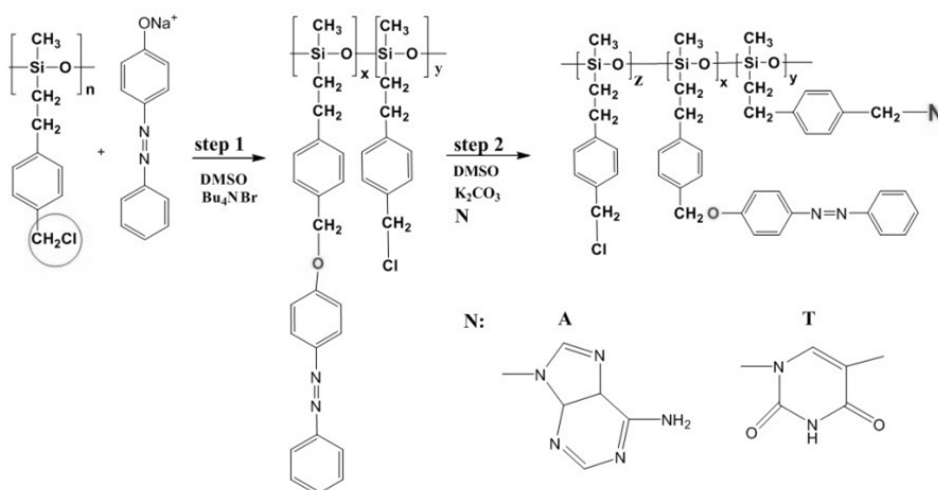
Fig. 1 – Polymer synthesis reaction scheme.

2.2. Measurements

Azo-polymeric films were spin-coated from 1,1,2-trichloroethane solutions onto siliconized glass support coated with amino-silane. Film thickness was measured using a Bruker Dextak XT profilometer with Vision 64 interpreter software. The experiments were performed in the absence of visible light using an EASYDROP Shape Analysis System (KRÜSS) equipped with DSA 1 evaluation software (liquid drop -15 μL). Surfaces were scanned using an Agilent Technologies 5100 AFM Atomic Force Microscope by Tipping Mode, in air and water.

3. Results and Discussions

Linear polysiloxane modified with 4-phenilazo-phenol and different percentage of adenine and thymine were investigated. The synthesized azopolymers were modified with $5\% - 26\%$ nucleobases substitution degree. The molecular weights (M_n) of the polymers are situated in the range of 12560 to 14670. The glass transition temperature (T_g) values are located between $10°$ and $34°C$. Film thicknesses are situated between 500-600 nm. The main characteristics of synthesized polymers are listed in Table 1.

It was found that for a polysiloxane whose CBZ groups have been substituted 54% with 4-phenyl-azophenol the value of the contact angle increases by 7° compared to an unsubstituted polysiloxane. This indicates the presence of azobenzene groups on the film surface.

Table 1

Characteristics of the synthetized polymers

Sample code	Substituent 1 Substituent 2	Gs (%)	Mn	T_g (°C)	Contact angle, [°]
Linear polysiloxane	-	-	-	-36	83
CM 11	4-phenilazophenol	54	12560	10	90
CM 4	4-phenilazophenol adenine	50 5	14670	28	89
CM 9	4-phenilazophenol adenine	53 15	14650	23	92
CM 13	4-phenilazophenol thymine	54 13	14450	35	91
CM 8	4-phenilazophenol thymine	45 26	13660	34	90

Gs – substitution degree; T_g - glass transition temperature;

In the case of nucleobases chain insertion in the side chain, regardless of the percentage of nucleobase (5% – 26%) connected to the siloxane chain, it was found that the contact angle value is kept around the same rate 89° – 92°. Doubling the percentage of thymine in the side chain from 13% to 26% there is a slight decrease in the contact angle with only 2º, a value within the limit of measurement errors (± 2°). These aspects indicate that the nucleobases connected in the side chain are arranged inside the polysiloxanic chains.

These aspects can be correlated with the present literature (Hurduc *et al*., 2007; Epure *et al*., 2011) and confirm that polysiloxane with small percent of CBZ substituted groups takes the form of a cluster, with hydrophobic azobenzene groups located on the outside and thymine and adenine (hydrophilic) inward; thus, the value of the contact angle is dictated by the azobenzene groups.

To observe the material response mode under the influence of the aqueous media, we plot the contact angle evolution over one hour of monitoring (Fig. 2).

For adenine-functionalized azo-polysiloxanes, a linear increase in hydrophilicity is observed in the first 15 minutes followed by a plateau of constant values around 85°. In the case of thymine-functionalized azo-polysiloxanes, is observed a gradual decrease of hydrophobicity in steps. After one hour the contact angle values decrease with the increasing thymine percentage on azopolymeric chain. This reflects a more hydrophilic nature of thymine azopolymers compared with that with adenine. Is the case of sample CM 8 that in one hour presents a decrease of 12°. Even the contact angles values for azo-polysiloxanes modified with nucleobases are initially situated in the same range in this case thymine azo-samples present a faster decrease of hydrophobicity.
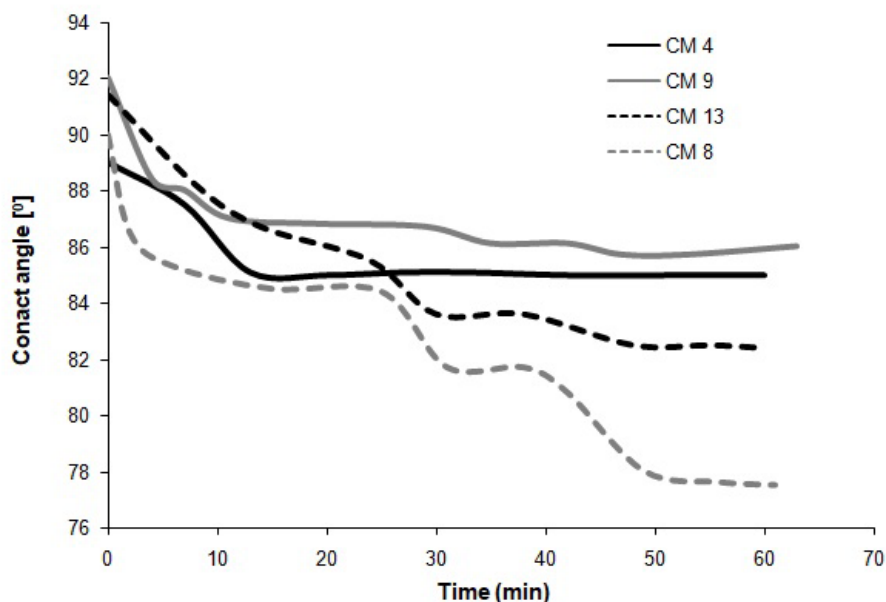
Fig. 2 – Mean observed contact angle evolution over one hour monitoring.

To observe what physically happens on the film surface we studied the behaviour of samples under aqueous media with the help of AFM technique. One-hour real-time recording of changes in polymeric surfaces under the influence of aqueous medium was performed by mounting a liquid cell on the AFM tray.

Studies show that nucleobase-modified azo-polysiloxanic surfaces present physical changes with the formation of different shapes islands, depending on the chemical structure of the polymer support. The process of azo-polysiloxanes reorganization under the influence of the aqueous environment is a rapid process that takes place in the first minutes and continues during the holding of the sample in water.

The first studied sample is CM 4 - polysiloxane modified with 50% 4-phenyl-azophenol and an insignificant amount of adenine (Fig. 3). After contact with the external environment, the appearance of very small horizontal material formations was observed. One explanation for this would be that at low degrees substitution of the polysiloxanes with azo-groups, the polymer chain adopts a cluster conformation where the hydrophilic CBZ groups are predominantly arranged on the cluster surface. Upon contact of the polymeric film surface with the aqueous medium, the free energy of the surface is disturbed and the system trying to regain its equilibrium state by reorganizing it.
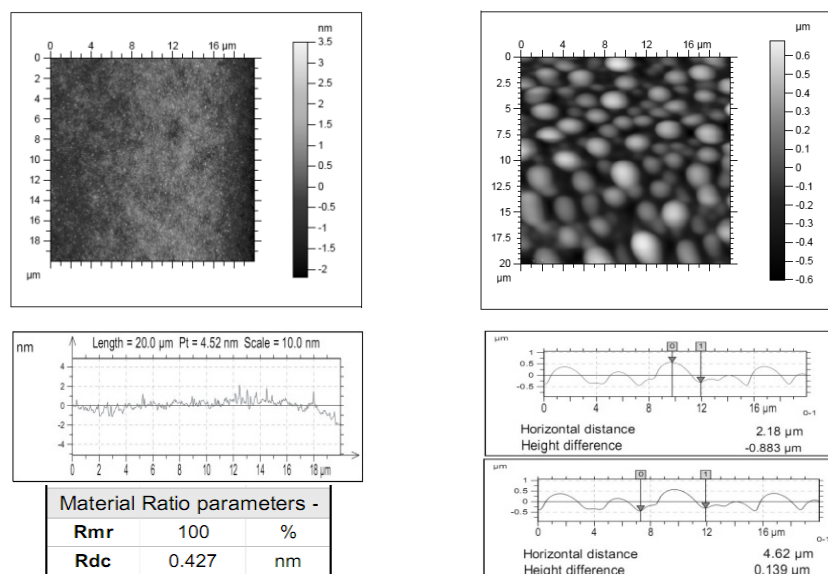
Fig. 3 – AFM images and surface geometry of the CM 4 sample in air (left) and under
the influence of the aqueous medium after 1h (right).

In the case of a higher percentage introduction of adenine on the chain
was observed that a series of aggregates appear on the film surface that do not
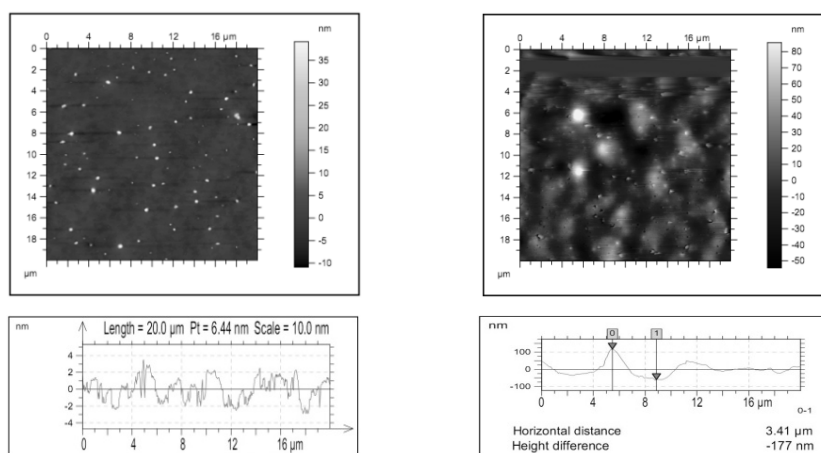influence the response of the material under the aqueous medium (Fig. 4).

Fig. 4 – AFM images and surface geometry of the CM 9 sample in air (left) and under
the influence of the aqueous medium after 1h (right).

Aggregates are formed due to associations between adenine-type groups, such associations being reported in the literature (Luo *et al*., 2001; Preuss and Bechstedt, 2008; Epure *et al.*, 2011). Introduction of adenine leads to polymer chain stiffening and stabilize the surface due to the voluminous structure, located towards the inside of the lump conformation of the polysiloxane chains. The azobenzene groups are predominantly to the outside of the cluster, as confirmed by the increased value of the contact angle (92°).

In the case of thymine chain introduction (sample CM 13) was observed a similar behaviour with sample CM 9, from the point of view of the surface reorganization aspect (Fig. 5). Upon contact with the aqueous medium, the surface of the polymeric film was reorganized into small material formations that reach 237 nm in height and approximately 6.91 μm in width after one hour. The evolution of the geometric characteristics in this case was much faster compared to modified azo-polysiloxane with the same percentage of adenine. We can say that thymine, being a pyrimidine base arranged inside the bundle allows an easier restructuring of azo-polymer chains.
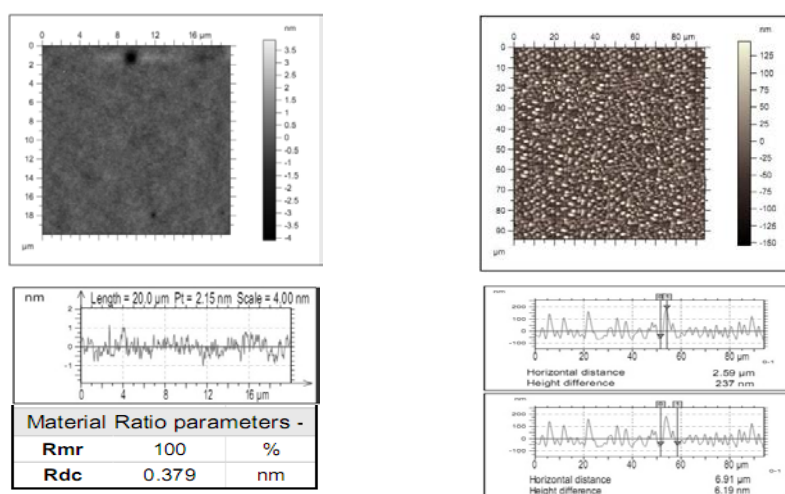

Fig. 5 – AFM images and surface geometry of the CM 13 sample in air (left) and under the influence of the aqueous medium after 1h (right).

The speed of surface reorganization increases with thymine percentage (from 13% to 26%). From the first minutes below the aqueous environment, the material formations reach heights over 750 nm (Fig. 6). It should be noted that the shape of the material following the material reorganization was the same for all azo-polysiloxane samples modified with nucleobases, regardless their percentage.
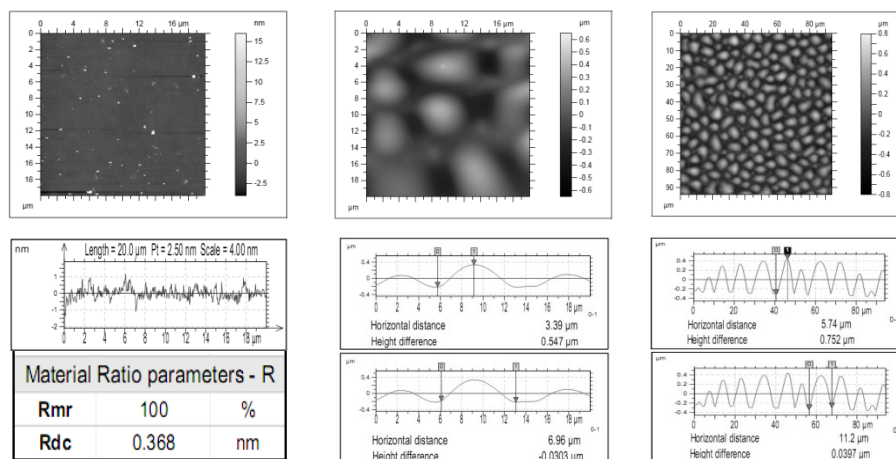
Fig. 6 – AFM images and surface geometry of the CM 8 sample in air (left), after first minutes (middle) and after 1h (right), under the influence of the aqueous medium.

Table 2 summarizes some of the characteristics of films studied under the influence of the aqueous environment.

Table 2
Surface modification of samples under aqueous media

Sample code	Surface roughness (nm)	Contact angle [°]			*first minutes	*after 1h
		time 0	after 1h	difference		
CM 4	0.427	89	85	4	*336/1.12*	883/4.62
CM 9	2.40	92	86	6	177/ -	170/5.00
CM 13	0.379	91	82	9	-	237/6.19
CM 8	0.368	90	78	12	547/6.96	752/11.2

* height difference (nm)/horizontal difference (μm)

It was observed that the roughness of the film does not affect, in this case, the contact angle value. The difference in roughness between the modified thymine and adenine samples is due to the existence of aggregates on the surface of the adenine film. From the studied samples, CM 9 present the lowest geometric characteristics so far, the islands of material formed after the reorganization of the film surface under the aqueous environment registering a height of 170 nm after one hour, due to steric effects mentioned earlier.

The degree of polymeric material reorganization under the influence of the aqueous environment depends on the percentage of nucleobase introduced on the side chain, starting from formations with very small vertical dimensions

(CM 9 – 177 nm) and reaching large sizes of 11.2 μm but spread over a narrower horizontal area (CM 8). A directly proportional relationship can be observed between the hydrophilicity and material islands formed. They both increase in contact with water. This leads us to assumption that the mechanisms of surface wetting could be absorption and spreading. The possible correlation between these two phenomena and the topography of the biopolymer surfaces is the subject of an ongoing research.

4. Conclusions

For nucleobase-modified azo-polysiloxanes, it was found that the type and percentage of nucleobase introduced into the side chain does not change the contact angle values. The contact angle evolution over one hour monitoring reflects a more hydrophilic nature of thymine azopolymers compared with that with adenine. Even the contact angles values for azo-polysiloxanes modified with nucleobases are initially situated in the same range in this case thymine azo-samples present a faster decrease of hydrophobicity.

AFM studies under water show that nucleobase-modified azo-polysiloxanic surfaces present physical changes with the formation of different shapes islands, depending on the chemical structure of the polymer support. The process of azo-polysiloxanes reorganization under the influence of the aqueous environment is a rapid process that takes place in the first minutes and continues during the holding of the sample in water. This study revealed that adenine introduction on polymeric chain increases its rigidity, the steric effects, and can induce or stabilize the surface. The presence of thymine on the chain it has the opposite effect, assisting to an increase in the speed of surface reorganization also correlated with increasing hydrophilicity.

Even now are preferred stabile materials in cell development application, this class of polymers can bring new opportunities to study cell behaviour on dynamic surfaces without the help of laser irradiation.

REFERENCES

Amo-Ochoa P., Zamora F., *Coordination polymers with nucleobases: From structural aspects to potential applications*, Coordination Chemistry Reviews, **276**, 34-58 (2014).
Cheng C.C., Huang C.F., Yen Y.C., Chang F.C., *A "plug and play" polymer through biocomplementary hydrogen bonding*, J. Polym. Sci. Part A Polym. Chem., **46**, 6416-6424 (2008).
Epure E.-L., Taran E., Hurduc N., Moleavin I.A., Nguyen A.V., Nichita N., *Azo-polymers modified with nucleobases and their interactions with DNA molecules*, Polymer Bulletin, **67**, 467-478 (2011).

Hua Z., Pitto-Barry A., Kang Y., Kirby N., Wilks T.R., O'Reilly R.K., *Micellar nanoparticles with tuneable morphologies through interactions between nucleobase-containing synthetic polymers in aqueous solution*, Polym. Chem., **7**, 4254-4262 (2016).

Hurduc N., Enea R., Scutaru D., Sacarescu L., Donose B.C., Nguyen A.V., *Nucleobases modified azo-polysiloxanes, materials with potential application in biomolecules nanomanipulation*, J. Polym. Sci. A, **45**, 4240-4248 (2007).

Hurduc N., Macovei A., Păiuş C.-M., Raicu A., Moleavin I., Branza-Nichita N., *Azo-polysiloxanes as new supports for cell cultures*, Materials Science and Engineering: C, **33** (4), 2440-244 (2013).

Imaz I., Rubio-Martinez M., An J., Sole-Font I., Rosi N.L., Maspoch D. *Metal–biomolecule frameworks (MBioFs)*, Chem. Commun., **47**, 7287-7302 (2011).

Karageorgiev P., Neher D., Schultz B., Stiller B., Pietsch U., Giersieg M., Brehmer L., *From anisotropic photo-fluidity towards nanomanipulation in the optical near-field*, Nat. Mater., **4**, 699-703 (2005).

Li J., Wang Z., Hua Z., Tang C., *Supramolecular nucleobase-functionalized polymers: synthesis and potential biological applications*, J. Mater. Chem. B, DOI: 10.1039/C9TB02393C (2020).

Luo R., Gilson H.S.R., Potter M.J., Gilson M.K., *The Physical Basis of Nucleic Acid Base Stacking in Water*, Biophysical Journal, **80**(1), 140-148 (2001).

Lutz J.-F., *Sequence-Controlled Polymers*, Wiley-VCH Verlag GmbH & Co. KGaA, 17-19 (2018).

McHale R., O'Reilly R.K., *Nucleobase containing synthetic polymers: Advancing biomimicry via controlled synthesis and self-assembly*, Macromolecules, **45**, 7665-7675 (2012).

Narita M., Hoshino F., Mouri M., Tsuchimori M., Ikawa T., Watanabe O., *Photoinduced immobilization of biomolecules on the surface of azopolymer films and its dependence on the concentration and type of the azobenzene moiety*, Macromolecules Polym. Bull., **40**, 623-629 (2007).

Pack D.W., Hoffman A.S., Pun S., Stayton P.S., *Design and development of polymers for gene delivery*, Nat. Rev. Drug Discov., **4**, 581-593 (2005).

Păiuş C.-M, Macovei A., Branza-Nichita N., Rocha L., Hurduc N., *Nanostructured azo-polysiloxanic films for biological applications*, Environmental Engineering and Management Journal, **11**(11), 2029-2034 (2012).

Preuss M., Bechstedt F., *Self-assembly of adenine-dimer chains on Cu(1 1 0): Driving forces from first-principles calculations*, Surface Science, **602**(9), 1643-9 (2008).

Rocha L., Păiuş C.M., Luca-Raicu A., Resmerita E., Rusu A., Moleavin I.A., Hamel M., Nichita N., Hurduc N., *Azobenzene based polymers as photoactive supports and micellar structures for biological applications,* Journal of Photochemistry and Photobiology A Chemistry, **291**, 16-25 (2014).

Ruckenstein E., Gourisankar S.V., *Surface restructuring of polymeric solids and its effect on the stability of the polymer-water interface*, Journal of Colloid and Interface Science, **109**(2), 557-566 (1986).

Sedlak M., Simunek P., Antonietti M., *Synthesis and N-15 NMR characterization of 4-vinylbenzyl substituted bases of nucleic acids*, J. Heterocyclic Chem., **40**, 671-675 (2003).

Spijker H.J., Dirks A.J., van Hest J.C.M., *Synthesis and assembly behavior of nucleobase-functionalized block copolymers*, J. Polym. Sci. Part A Polym. Chem., **44**, 4242-4250 (2006).

Stylianou K.C., Warren J.E., Chong S.Y., Rabone J., Bacsa J., Bradshaw D., Rosseinsky M.J., *CO₂ selectivity of a 1D microporous adenine-based metal–organic framework synthesised in water*, Chem. Commun., **47**, 3389-3391 (2016).

Voisin M., Ball M., O'Connell C., Sherlock R., *Osteoblasts response to microstructured and nanostructured polyimide film, processed by the use of silica bead microlenses,* Nanomedicine: Nanotechnology, Biology, and Medicine, **6**, 35-43 (2010).

Yager K.G., Barett C.J., *All-optical patterning of azo polymer films*, Curr. Opin. Solid State Mater, Sci. **5**, 487-494 (2001).

Yang H., Xi W., *Nucleobase-Containing Polymers: Structure, Synthesis, and Applications*, Polymers (Basel), Dec. **9**(12), 666 (2017).

COMPORTAMENTUL INDUS DE MEDIUL APOS A SUPRAFAŢEI FILMELOR AZOPOLISILOXANICE MODIFICATE CU NUCLEOBAZE

(Rezumat)

Ţinând cont de utilizarea filmelor azopolisiloxanice modificate cu nucleobaze ca suporturi pentru culturile celulare dar şi pentru aplicaţii privind imobilizarea biomoleculelor (în special ADN, ARN) care presupune contactul cu mediul apos, s-a realizat un studiu al proprietăţilor de suprafaţă ale acestor compuşi, prin evaluarea unghiului de contact al suprafeţei uscate şi în contact cu apa timp de o oră. Acest studiu a fost completat şi corelat cu studiile comportamentale AFM ale suprafeţelor sub influenţa mediului apos. Probele studiate prezintă o suprafaţă dinamică ce poate fi controlată, rezultatele indicând o reorganizare a lanţurilor polimerice funcţie de structura chimică.