Biocide properties of polyethylene films with sulfonamide moiety on the surface

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New azidobenzenesulfonamides were synthesized and their photochemical properties were investigated. Surface modification of polyethylene (PE) films by using new azidobenzenesulfonamides was carried out. The wetting angles for three liquids of different polarity (water, formamide and diiodomethane) have been studied to estimate the surface modification performed. The received values of wetting angles for determining the surface free energy γ_S (γ), as well as its components – polar γ_s^p, dispersive γ_s^d by the Owens-Wendt and Lifshitz-van der Waals γ^{LW}, acid-base γ^{AB} by the Lifshitz-van der Waals/acid-base methods. Antimicrobial activity of PE films with sulfonamide moiety was screened in vitro in the following bacterial cultures: Staphylococcus aureus, Escherichia coli, Mycobacterium luteum, Cystopteries tenuis. Preliminary screening of PE films with sulfonamide moiety revealed their inhibitory action against some kinds of bacteria.

1. Introduction

Surface modification is a convenient and sometimes the only way of polymer materials acquiring necessary properties since it does not change the polymer structure in mass. The development of modern technologies enables us to create polymer materials with a number of properties which would be impossible to realize in a single polymer. A polymer can acquire special properties either in the course of inter-chain polymer alloying by adding certain monomer units to the basic monomer being polymerized [1] or by modifying the ready-made sample [2-4].

Designing the structure of modified surface by various biologically active groups is one of the issues of current importance in present day polymer chemistry.

The chemistry of azides and nitrenes has attracted the attention of chemists since the discovery of phenyl azide by Griess [5] and the first proposal of nitrenes as reactions intermediates by Tiemann. Since then, many derivatives have been the subject of extensive investigation because of their important biological and industrial applications as photoaffinity labeling agents [6-7], cross-linking reagents in photoresists [8], the formation of conducting polymers [9], and the light induced activation of polymer surfaces [10-11]. In the other hand, it is well know that sulfonamides are widely used for therapeutic and prophylatic purpose in humans and other animals and sometime as additives in animal feed [12]. Therefore the aim of our work was syntheses new sulfonamides and modeling compounds based on them with biological active groups. The ability of azides to the photochemical decomposition with resulting active biradical can be used for modification of the polymer surface [13, 14-16]. In our work, as a polymer surface have been used PE films. It is well-know that PE is one of the more widespread polymers used for industrial and biomedical applications due to its special properties which include low density, flexibility and high chemical resistance [17-19]

In this article, we turn attention to the investigation of influence different moiety on the biocide property of polymer surface. For this purpose, the azidobenzenesulfonamides have been synthesized and biologically active compounds introduced in a polymer surface in order to obtain pharmacologically active properties.

2. Experemental part

2.1. Materials

4-aminobenzenesulfonamide (Aldrich) were used without further purification. Ethanol were of analytical grade, dried and purified before use. Diiodomethane, formamide (Fluka) are spectroscopic grade and used as received.

2.3. Characterization techniques

^{1}H NMR (400 MHz) spectra were recorded on a "Mercury-400" spectrometer using DMSO-d_6 as solvent. Chemical shifts are in ppm from the internal standard tetramethylsilane (TMS).

The IR spectra were obtained on a UR-20 spectrometer in KBr.

FT-IR-ATR spectroscopy was done over a range of 4000-800 cm^{-1} at room temperature.

All measurements of contact angles are advancing angles and were performed with a KRUSS G 10 contact angle measuring instrument. On each sample at least four

different locations were measured and results were averaged.

2.4. Synthesis of azidobenzenesulfonamides

N-(3-acetylphenyl)-4-azidobenzenesulfonamide (**aI**). The mixture of concentrated hydrochloric acid (2 ml) and water (10 ml) was added to a solution of 0.95 g (0.00327 mol) of *N*-(3-acetylphenyl)-4-aminobenzenesulfonamide in 5 ml of ethanol. The reaction mixture was stirred for 15 min at 0°C and a solution of 0.225 g (0.00327 mol) of NaNO₂ in 5 ml of water was added dropwise to the solution, keeping the temperature of the reaction mixture -5 - -10°C. The reaction mixture was stirred for 30 min at -5 °C. The cooled solution of 0.6376 g (0.00981 mol) of NaN₃ in 3 ml of water was added dropwise to the reaction mixture. After this, the reaction mixture was stirred for 1 h at room temperature. The organic material was extracted. The product was recrystallized from ethanol, m.p. 135°C (white crystals), yield 53%.

^{1}H NMR (400 MHz, DMSO-d₆), *%* (ppm): 7.18 (d, Ar, 2*H*), 7.76 (d, Ar, 2*H*), 3.84 (s, CH₃, 3*H*), 10.44 (s, SO₂NH, 1*H*). UV-VIS (ethanol) λ_{max}: 265 nm. IR (KBr, cm⁻¹): 2100, 1630, 1580, 1500, 740.

N-(1-naphthalenyl)-4-azidobenzenesulfonamide (**aII**). **aII** was prepared by the route of **aI** preparation. The light yellow crystals were collected by filtration and recrystallized from ethanol. Yield 48%, m.p. 138°C.

^{1}H NMR (400 MHz, DMSO-d₆), *%* (ppm): 7.15 (d, Ar, 2*H*), 7.40 (d, Ar, 2*H*), 10.08 (s, SO₂ NH, 1*H*), 7.14 – 8.04 (m, 7*H*). UV-VIS (ethanol) λ_{max}: 264 nm. IR (KBr, cm⁻¹): 2080, 1580, 1270.

4-azidobenzenesulfonamide (**aIII**). This compound was obtained according to the method described for **aI**, m.p. 145°C (white crystals), yield 63%. The product was recrystallized from ethanol.

^{1}H NMR (400 MHz, DMSO-d₆), *%* (ppm): 7.18 (d, Ar, 2*H*), 7.83 (d, Ar, 2*H*), 7.21 (s, SO₂ NH₂, 2*H*). UV-VIS (ethanol) λ_{max}: 260 nm. IR (KBr, cm⁻¹): 2100, 1600.

The general structures of azidosulfonamides is shown in the Scheme 1.
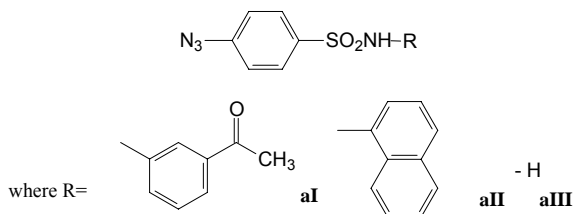
Scheme 1. The chemical structures of azidobenzenesulfonamides.

2.5. Photochemical properties of azidobenzenesulfonamides

Phenyl azides are known to undergo the photo-reaction under UV irradiation. The reaction of photolysis with formation active nitrenes (either singlet or triplet) has been observed [20]. Singlet phenylnitrene is primary reactive intermediate formed upon the photolysis of phenyl azide. Although triplet phenylnitrene, as well as didehydroazepine, has been directly observed by means of IR and UV spectroscopy in matrices and in solutions and the 3*H*-azepine derivative, respectively [21-22]. Nitrenes possess high reaction ability and take part in photoreactions.

The photolysis process with strong spectral changes which took place during UV irradiation (313 nm) of the solution of **aI**, **aII** and **aIII** in ethanol was shown in Fig. 1. A strong decrease of the absorption in the region in 245-290 nm (**aI**), 250-300 (**aII**), 238-292 (**aIII**) and their increase in the region 295-400 nm (**aI**), 220-235 nm (**aIII**), 230-250 nm, 308-385 nm (**aII**) were observed.
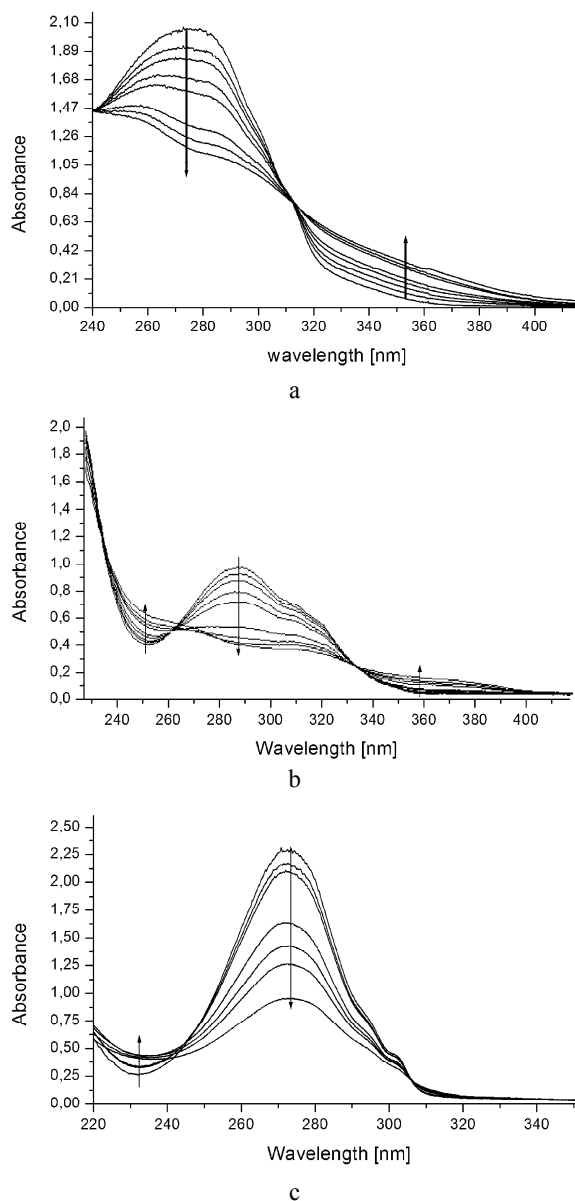

Fig. 1. The changes of the absorption spectrum of aI (a), aII (b) and aIII (c) in ethanol (C = 10⁻⁵ mol/L) during UV-irradiation (313 nm).

In order to investigate photoactive abilities of new azidobenzenesulfonamides photolysis quantum yields were determined. High photochemical activity of azidobenzenesulfonamides was confirmed by the received results **aI** φ= 0.48 and for **aIII** φ= 0.51. In the case of **aII** more low value of photolysis quantum yield (φ=0.14) was explained by existence of effective singlet-triplet and triplet-triplet conversion of the excitation energy on the naphthalene π-electron system. This was described with details previously [23].

2.6. Modification of PE films

In the present study, we used polyethylene (PE) films which were modified by a procedure outlined in Ref [13]. The possibilities of surface photo-modification of PE films LDPE (GGNX 18 D003) by new azides containing biological active sulfonamide group **aI**, **aII**, **aIII** have been studied. PE films (size 4×7 cm) were prepared. Polymer films were sheeted by 1% azidobenzenesulfonamides using acetone as solvent and dry samples were irradiated by UV lamp DRT-1000 during 30 minutes at 20°C. The properties of modified polymer surface were established by IR- spectroscopy and direct contact angle measurement for standard liquids: diiodomethane, formamide and water, using Kruss G-10 instrument [13].

The Owens-Wendt [24] and Lifshitz-van der Waals/acid-base [25] approaches were used to estimate values of surface free energy γ_S (mJ/m^2) and its components – polar γ_s^p and dispersive γ_s^d of the polyethylene surfaces. The data of three test liquids used to determine the surface free energy of PE are shown in Table 1. All liquids were provided in an analytical-reagent quality. Approximately 70 contact angles were determined for each drop.

Table 1. The values of surface tension and their constituent γ_L^d, γ_L^p (mJ/m^2) for testing liquid (at 20°C)

Liquid	γ_L	γ_L^d	γ_L^p
Diiodomethan	50.8	50.8	0
Formamide	58.0	39.0	19.0
Water	72.8	21.8	51.0

FT-IR-ATR (PE films after irradiation): The important bands in examining degradation mechanisms include the broad υ (C=C) between 1585 and 1600 cm^{-1}, δ (C-H) at 1000 cm^{-1}, δ (SO$_2$N) at 1360 and 1280 cm^{-1}, υ (C=O) at 1676 cm^{-1}, δ (C-H) at 840 cm^{-1}.

2.7. Biocide properties of PE films

In the sterile "Petri dishes" have been added 2% solutions agar media (beef-extract agar). The media cooled and after the solidification have been added the modified PE films. The surface of the films have been treated by bacterial suspensions with 0,7 % solutions of nutrient agar. The plates were incubated at 37ºC for samples with bacteriums. The times of incubation 36 h. The reading was carried out by measuring the % death of microorganisms during the contact with PE modificated surfaces. The antibactericidal screening was done for bacteries namely Staphylococucus aureus, Escherichia coli, Mycobacterium luteum, Cystopteries tenuis. The dates of death of microorganisms in comparision with non modified films are presented in the Table 2.

Table 2. Biocide properties of modified films.

The modified PE film	The death of microorganisms in relation to non modified film, %			
	E.coli	St.aureus	Myc.luteum	C.tenuis
aI	25	13	30	9
aII	30	34	90	35
aIII	50	49	90	27

As we can see the films, which were modified by introduction azides **aII** and **aIII**, can be characterized by good bactericidal properties against all microorganisms.

3. Results and discussion

The syntheses have proceeded in two stages: preparation of the sulfonamides and azides based on them. New azidobenzenesulfonamides were synthesized by diazotization of corresponding amines and treatment by diazonium salt with aqueous solution of sodium azide [26]. The additional procedure of surface photo-modification of PE was used.

Young's equation (1) allows a calculation of the stationary wetting angle of contact and describes the equilibrium of forces between the surface tensions at the 3-phase boundary [27].

$$\gamma_S = \gamma_{SL} + \gamma_L \times \cos\theta \qquad (1)$$

γ_S and γ_{SL} cannot be determined indirectly through experiments either, γ_S is not a clearly defined physical parameter. In order to determine the surface energy of solids through a measurement of the contact angle, Owens-Wendt theoretical statement describing the interfacial tension γ_{SL} have been used.

Owens and Wendt set up that surface free energy possessed two components: polar and dispersive

$$\gamma_S = \gamma_S^d + \gamma_S^p$$ and the following equation was proposed:

$$\gamma_{SL} = \gamma_S + \gamma_L - 2\sqrt{\gamma_L^d \times \gamma_S^d} - 2\sqrt{\gamma_L^p \times \gamma_S^p} \qquad (2)$$

where γ_S - surface free energy of polymer surface in vacuum, γ_L - surface free energy of testing liquid, γ_L^d - dispersive component of surface free energy of testing liquid, γ_L^p -polar component of surface free energy of testing liquid, γ_S^d - dispersive component of surface free energy of polymer surface, γ_S^p -polar component of surface free energy of polymer surface.

Including Young equation such formula can be taken out:

$$\gamma_L \frac{1 + \cos\theta}{2} = \sqrt{\gamma_S^d \times \gamma_L^d} + \sqrt{\gamma_S^p \times \gamma_L^p} \qquad (3)$$

For γ_S determination from equation (3) it is necessary to measure angle θ at least for two various liquids with known values of γ_L^d and γ_L^p, and then to solve the system of equations. One of this liquids should be characterized by high value of γ_L^d and low value of γ_L^p and the second liquid - inversely. The most often, water and diiodomethane were used as the pair of testing liquids.

The values of surface free energy and its components for testing liquids used in contact angle measurements are collected in Table 1.

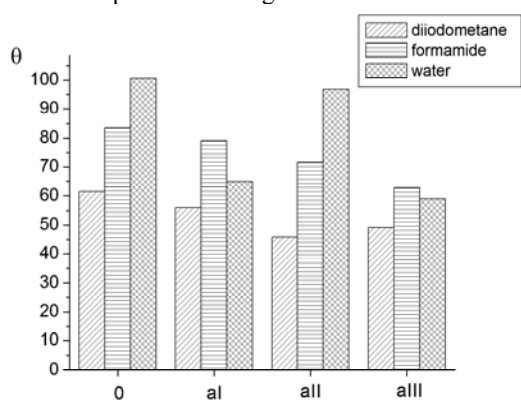The values of contact angle θ for experimental samples PE are presented in Fig. 2:

*Fig. 2. Contact angles for polyethylene- liquid: 0 - unmodified film; **aI, aII, aIII** - photo- modified samples by azidobenzenesulfonamides.*

The significant changes of contact angles for all samples after photochemical modification were observed. The surface free energy γ_S was calculated as the sum of its dispersive and polar components (Fig. 3).

PE films after modification by **aI** have a low surface energy 29.01 mJ/m² with a negligible polar component, by **aII** γ_S considerably increase 35.49 mJ/m². PE film which

was modified by **aIII** possessed a higher surface energy 42.94 mJ/m² with large polar component 15.55 mJ/m² (Fig. 3). The surfaces become more hydrophilic with increasing polar shares, i.e. the contact angle of water drops falls due to the better wetting. The effects can be explained as a consequence of the photochemical modification of the surface. High energy values are favourable for surface wetting.
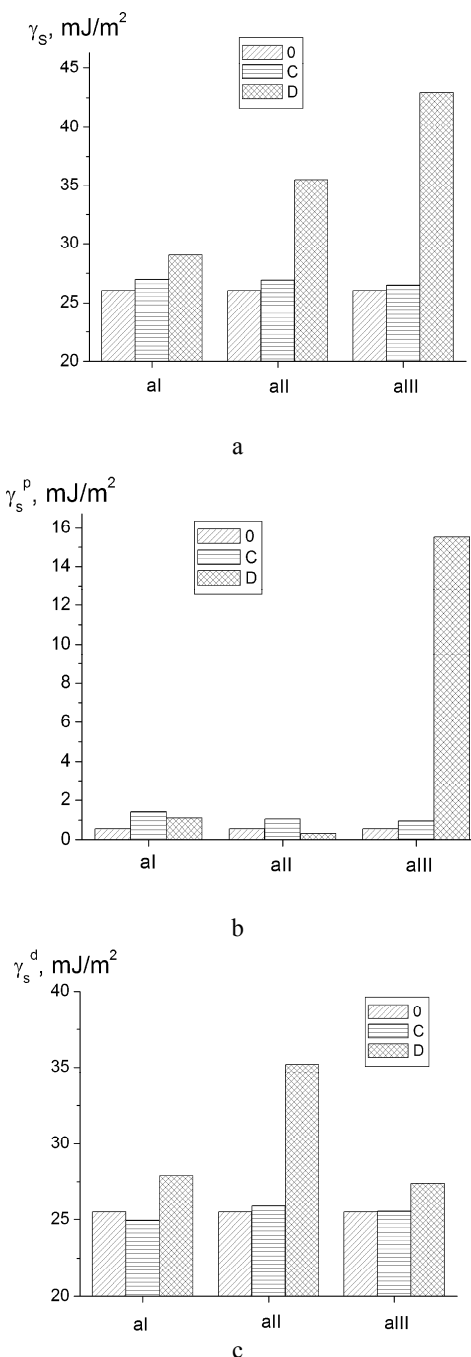
*Fig. 3. Surface free energy a) of PE films calculated by Owens-Wendt approach and its: b) – polar (γSp); c) – dispersive (γSd) shares; 0 - unmodified film, C - unirradiated, D - photo-modified samples by azidobenzenesulfonamides **aI, aII, aIII**.*

The calculations according to basic-acid Van Oss-Good theory were realized to check surface polarity. Surface free energy can be presented as sum of two components: $\gamma_i = \gamma_i^{LW} + \gamma_i^{AB}$.

γ_i^{LW} is connected with all long range interactions such as dispersive, polar and inductive interactions. γ_i^{AB} results from acid-basic interactions. Index i can take values from natural number set and the following surface or testing liquids are signed by it.

According to this theory chemical substances can be divided into: bipolar (with properties suitable both for Lewis bases and for Lewis acids), monopolar (with properties characteristic either for Lewis base or Lewis acid) and apolar (neither Lewis base nor Lewis acid properties).

For bipolar substances $\gamma_i^{AB} = 2(\gamma_i^+ \times \gamma_i^-)^{1/2}$, where γ^+ signed γ^{AB} component corresponding to Lewis

acid surface free energy and γ^- signed γ^{AB} component corresponding to Lewis base.

For mono- and apolar substances $\gamma^{AB} = 0$. Three components: γ_S^{LW}, γ_S^+ and γ_S^- should be known to calculate surface free energy γ_S of tested material. These components can be determined from the results of contact angle examinations. Tested surface should be wetted by three various liquids (these values γ_{Li}^{LW}, γ_{Li}^+ and γ_{Li}^- should be find). Then, γ_S can be calculated from system of three equations:

$$\sqrt{\gamma_S^{LW} \times \gamma_{Li}^{LW}} + \sqrt{\gamma_{Li}^+ \times \gamma_{Li}^-} + \sqrt{\gamma_S^+ \times \gamma_S^-} = \gamma_{Li}\frac{1+\cos\theta}{2} \quad (4)$$

The calculation results for tested surfaces are presented in Table 3.

Table 3. The values of surface free energy γ (mJ /m^2) of PE films calculated by acid-base approach and its constituent γ^{LW} and γ^{AB} (0- unmodified film, C- unirradiated, D- photo-modified samples by azidobenzenesulfonamides aI, aII, aIII)

Sample	γ^{LW}		γ^{AB}		γ^+		γ^-		γ	
	C	D	C	D	C	D	C	D	C	D
0	27.72		1.32		0.20		2.15		29.05	
aI	28.09	30.83	2.50	1.97	0.32	0.25	4.86	3.83	30.59	32.81
aII	27.83	36.56	0.72	0.31	0.05	0.03	2.70	0.81	28.55	36.88
aIII	28.83	34.74	2.88	7.21	0.50	0.35	4.15	36.87	31.71	41.95

The received values of the surface free energy γ, as well as its components Lifshitz-van der Waals γ^{LW}, acid-base γ^{AB} by the Lifshitz-van der Waals/Van Oss-Good (acid-base method) are in a good accordance with Owens-Wendt approach (Table 3).

High polarity of polyethylene surface modified by azide **aIII** was confirmed by the values of surface free energy determined by Van Oss-Good method and also by results received by Owens-Wendt method. The electronodonor character of surface was proved by high part of γ^- component, corresponding to Lewis base. At the same time, high part of γ^{LW} component in surface free energy, connected with all long range interactions should be underlined.

For microbiological analysis of the modified films, bacteria *Staphylococcus aureus* , *Escherichia coli*, and *Mycobacterium luteum, Cystopteries tenuis* were chosen.

The statistical analysis of the results showed that the antimicrobial properties of modified films were significantly more effective in comparision with non modified film. All the modified films, demonstrated a hight activity against bacteria (*Mycobacterium luteum*) in comparision with non modified film. It is important to note that the activity of the modified films was different for different type of bacteries. A modified PE film by

aIII contains a –NH$_2$ group in the structure and more active than others samples against bacteria. The same principle of the structure–activity relationship for other models was observed in research [21].

4. Conclusions

The experimental procedures were standardized for obtaining optimum yield. The modified PE films with anti-microbial activity have been prepared. All sullfonilamides derivatives were characterized by NMR- and IR- spectroscopy. The investigations of photochemical properties have been shown ability new azidobenzenesulfonamides for polymer surface modification. The hydrophilic character of surface was received by using azidobenzenesulfonamides as modifier. During photo-modification process only surface properties of modified product, not polymer structure in mass, was changed. In all cases PE films modified with azides were characterized by increased values of γ_S.

The modified PE films by **aI**, **aII**, **aIII** are characterized using a higher antimicrobial activity *in vitro* than the initial non modified PE film. The PE surface modified by **aIII** shown higher antimicrobial activity in comparison with PE surfaces modified by **aI** and **aII** against *Escherichia coli in vitro.*

The achieved results confirmed, that modification of polymer surfaces by azides based on benzenesulfonamides enables to use them as perspective materials, for example as biostabilizers for plastic masses.

Acknowledgement

References

[1] O. Krupka, A.Yu. Kolendo, K. Kushnir, J. Blazejowski, Mol. Cryst. Liq. Cryst. **427**, 233 (2005).

[2] J. Sacristan, H. Reinecke, C. Mijangos, J. Polymer. **41**, 5577 (2000).

[3] J. Spanring, C. Buchgraber, M.F. Ebel, R. Svagera, W. Kern, J. Polymer. **47**, 156 (2006).

[4] X. Jia, M. Herrera-Alonso, T. McCarthy, J. Polymer. **47**, 4916 (2006).

[5] P. Griess, Philos. Trans. R. SOC. **13**, 377 (1864).

[6] S. X. Cai, D. J. Glenn, K. R. Gee, M. D. Yan, R. E. Cotter, N. L. Reddy, E. Weber, J. Keana, Bioconjugate Chem. **4**, 545 (1993).

[7] H. Bayley, J. Staros, In Azides and Nitrenes – Reactivity and Utility, Academic Press Inc. New York (1984).

[8] S. X. Cai, D. J. Glenn, M. Kanskar, M. N. Wybourne, J. F Keana, Chem. Mater. **6**, 1822 (1994).

[9] E. W. Meijer, S. Nijhuis, F. C. Vroonhoven, J. Am. Chem. Soc. **110**, 7209 (1988).

[10] P. Nahar, N. Wali, R. Gandhi, Anal. Biochem. **294**, 148 (2001).

[11] S. X. Cai, D. J. Glenn, J. F. Keana, J. Org. Chem. **57**, 1299 (1992).

[12] A. R. Long, L. C. Hsieh, M. S. Malbrough, C. R. Short, S. A. Barker, J. Agric. Food Chem. **38**, 423 (1990).

[13] K. Golec, O. Krupka, V. Smokal, M. Wilczek, M. Kostrzewa, V. Syromyatnikov, Mol. Cryst. Lyq. Cryst. **497**, 323 (2008).

[14] E. Kenawy, F.I. Abdel-Hay, Reactive and Functional Polymers. **66**, 419 (2006).

[15] J. Khandare, T. Minko, Progress in Polymer Science. **31**, 359 (2006).

[16] L. G. Donaruma, J. R. Dombroski, J. Med. Chem. **14**, 460 (1971).

[17] L. Fang, Y. Leng, P. Gao, Biomaterials. **26**, 3471 (2005).

[18] J. Wang, N. Huang, P. Yang, Y. X. Leng, H. Sun, Z. Y. Liu, P. K. Chu, Biomaterials. **25**, 3163 (2004).

[19] H. Lee, S. Hong, K. Yang, K. Choi, Microelectron. Eng. **83**, 323 (2006).

[20] M.-L. Tsao, M. S. Platz, J. Am. Chem. Soc. **125**, 12014 (2003).

[21] J. C. Hayes, R.S. Sheridan, J. Am. Chem. Soc. **112**, 5879 (1990).

[22] Y.-Z. Li, J. P. Kirby, M. W.George, M. Poliakoff, G. B. Schuster, J. Am. Chem. Soc. **110**, 8092 (1988).

[23] O. Krupka, A. Kolendo, A. Doroshenko, J. Vop. Khim.Tekh. Ukr. **2**, 103 (2005).

[24] D. K. Owens, R.C. Wendt, J. Appl. Polym. Sci., **13**, 1741 (1969).

[25] D. Y. Kwok, D. Li, A. W. Neumann, Langmuir, **10**, 1323 (1994).

[26] P. A. Smith, J. H. Hall, J.Am.Chem.Soc. **84**, 480 (1962).

[27] T. Young, Phil. Trans. Roy. Soc. London, p. 255. (1805).

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