

*Dedicated to Professor Claude Nicolau
on the occasion of his 80th anniversary*

INVESTIGATION ON THE PROPERTIES OF POLY (2-HYDROXYETHYL METHACRYLATE -CO- 3,9-DIVINYL-2,4,8,10-TETRAOXASPIRO (5.5) UNDECANE) AS A FUNCTIONAL POLYMERIC SYSTEM

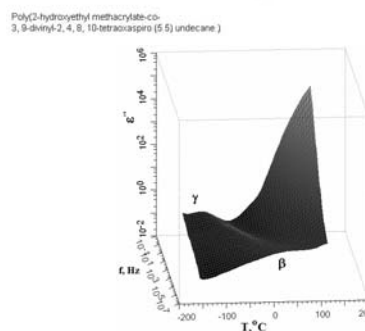
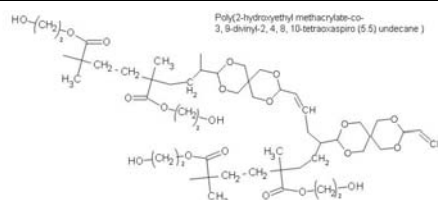
Vera BALAN,^{a,b} Mihai ASANDULESA,^a Elena BUTNARU,^a Aurica P. CHIRIAC,^{a*}
Nita TUDORACHI^a Loredana E. NITA,^a Iordana NEAMTU^a and Alina DIACONU^a

^a“Petru Poni” Institute of Macromolecular Chemistry 41-A Grigore Ghica Voda Alley, 700487 Iași, Roumania

^b“Gr.T. Popa” University of Medicine and Pharmacy, Faculty of Medical Bioengineering, 9-13 Kogalniceanu Street, 700715, Iași, Roumania

Received November 13, 2015

The aim of this study was to evaluate the physicochemical characteristics of a functional polymeric system based on poly (2-hydroxyethyl methacrylate) and 3, 9-divinyl-2, 4, 8, 10-tetraoxaspiro (5.5) undecane (U), in terms of molecular weight, temperature-sensitive abilities, thermal stability, rheological and dielectrical properties. The dependence of the copolymer particles size versus temperature highlighted the temperature-sensitive ability of the evaluated compound. DSC indicated that the copolymer exhibited a single glass transition temperature (T_g), an endothermic process and two degradation processes. Fluorescence spectroscopy confirmed the presence of the hydrophobic spiroacetal moiety in the copolymer, which confers specific conformational configuration to the copolymer. The dielectric properties of the copolymer have been evaluated in order to investigate the relaxation processes. It was found that the main contribution to dielectric relaxation derived from the non-cooperative motion of side groups only, or parts of them and no contribution from the main chains was noted.



INTRODUCTION

In recent years, the design of macromolecular systems with stimuli-sensitive abilities is of growing interest for various domains, including biomedical field, optics, electronics, etc.^{1,2} In the same time, the study of relationship between the

chemical and physical structure of the polymers and their properties is the key to further advance important applications. Owing to their remarkable properties, such as: gel formation capacity, binding properties, amphiphicity, good oxidative and thermal stability, biocompatibility, sensibility at pH and temperature, formers of good films and antioxidant

* Corresponding author: achiriac1@yahoo.com

character, polymeric systems based on acrylic monomers and 3, 9-divinyl-2, 4, 8, 10-tetraoxaspiro (5.5) undecane (U) may be included into the class of “smart” polymers.³⁻⁵ In this context, several representative studies were dedicated to the homopolymerization and copolymerization of 2-hydroxyethyl methacrylate owing to their multiple application fields, namely optical lenses, implants, drug delivery devices and support for enzyme immobilization.⁶⁻⁸ In particular, in a previous study, the synthesis of a new copolymer based on poly 2-hydroxyethyl methacrylate (PHEMA) and 3, 9-divinyl-2, 4, 8, 10-tetraoxaspiro (5.5) undecane (U) through radical polymerization procedure, in the presence of 2,2'-azobis (2-methylpropionitrile) was investigated.³

The incorporation of spiroacetal groups into the polymer structure could improve the adhesive properties⁹ and ensure intramolecular strategies for

coupling of various compounds.¹⁰ The interest for this type of copolymers is thoroughly justified taking into consideration the usefulness of these compounds in a wide range of applications. However, a great attention concerning their physiochemical characteristics should be devoted and more-in-depth studies are needed in order to fully exploit their potential. In this regard, this study evaluates the physiochemical characteristics of PHEMA_U copolymer in terms of molecular weight, temperature-sensitive abilities, thermal stability, rheological and dielectrical properties, for bringing useful information for the specific use of these compounds.

RESULTS AND DISCUSSION

The copolymer system has the following idealized structure presented in Fig. 1.

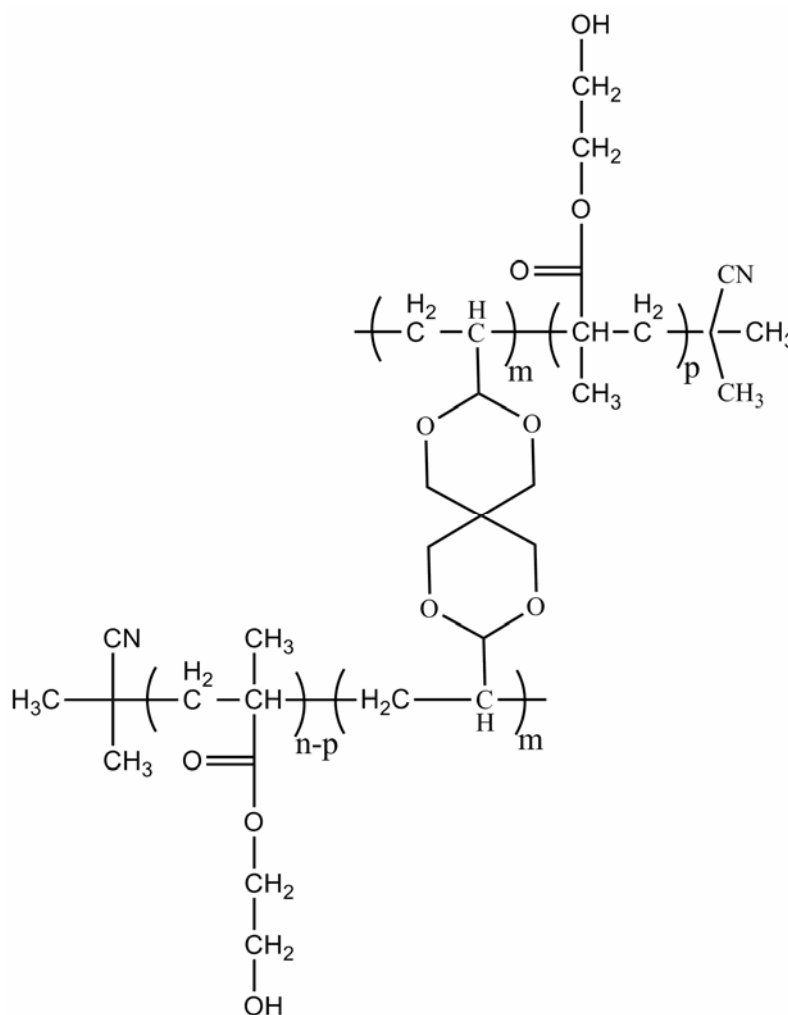


Fig. 1 – The idealized copolymer structure .

Molecular weight evaluation by SLS

Molecular weight is an extremely important variable since it relates to the physical properties of the material; the size of the molecules is dependent upon the ionic strength and its concentration in the solution.¹¹ The molecular weight of the synthesized copolymer was evaluated using a Zetasizer NanoS system that measures the intensity of the static light scattered (SLS) at one angle (173°) by the copolymer solutions, prepared at different concentrations. In Table 1 are presented the parameters used in the evaluation of the molecular weight. The intensity of SLS is proportional with the weight-average molecular weight (Mw) and the concentration of the sample. A relatively large size sample exhibit non-isotropic scattering profile with measured intensities that are dependent on the angle of observation. However, as the particle size decreases, the sample scattering becomes isotropic and the angular dependence of the measured

intensity is minimized (Fig. 2). The determined molecular weight of the synthesized copolymer was 22.05 ± 1.98 KDa.

Z-average (d, nm) vs. temperature

PHEMA_U solution behaviour studied versus temperature variation, in the range of 22-40 °C (Fig. 3), exhibited a first stage of an approximately constant hydrodynamic diameter, followed by a thermo-induced particle size change at 34 °C, where a minimum of 700 nm was registered; subsequently the hydrodynamic radius of the copolymer particles experienced a gradually increase as temperature raise towards 40 °C. This analysis indicated the sensitivity of the evaluated compound with the temperature, especially in the range between 31-37 °C. The thermo sensitive character could be attributed probably to the hydrophobic-hydrophilic balance of the copolymer.¹²

Table 1

The parameters used in the evaluation of the molecular weight by SLS

Concentration (g/ml)	Intensity (kcps)	Residual Intensity (kcps)	R ₀ (1/cm)	KC/R ₀ P (1/Da)	Molecular weight (KDa) 22.05±1.98
0.002	332	247	$8.482 \cdot 10^{-5}$	$5.382 \cdot 10^{-5}$	
0.004	452	367	$12.604 \cdot 10^{-5}$	$7.245 \cdot 10^{-5}$	
0.006	545	460	$15.797 \cdot 10^{-5}$	$8.670 \cdot 10^{-5}$	
0.008	632	547	$18.785 \cdot 10^{-5}$	$9.722 \cdot 10^{-5}$	
0.010	725	640	$21.979 \cdot 10^{-5}$	$10.387 \cdot 10^{-5}$	

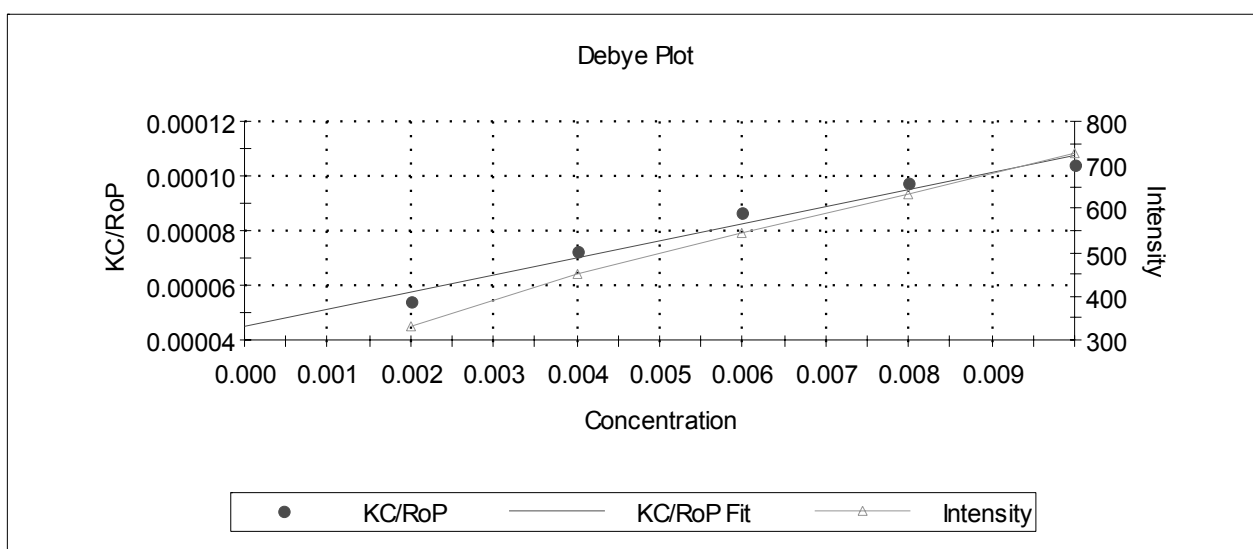


Fig. 2 – The dependence of KC/R₀P versus concentration for PHEMA_U solutions.

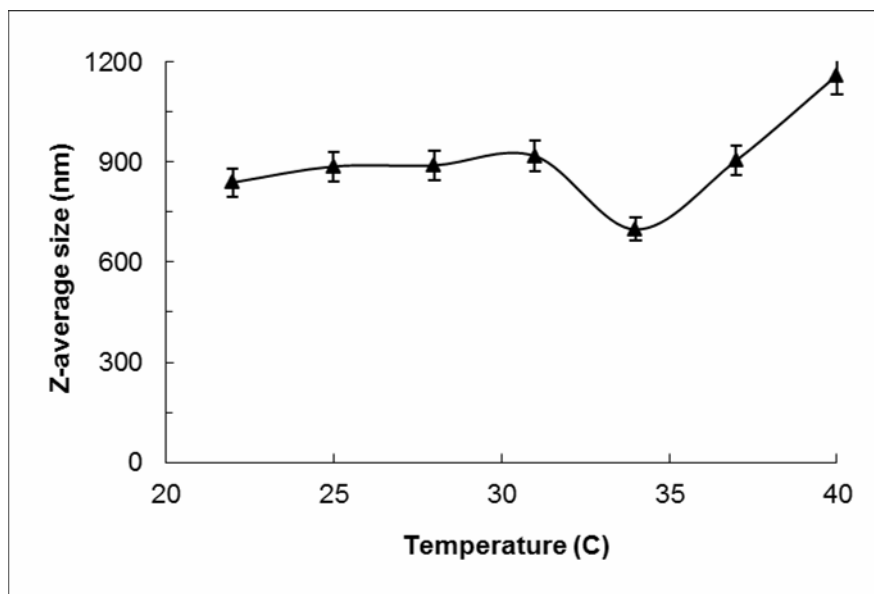


Fig. 3 – Hydrodynamic diameter of PHEMA-U vs temperature (the results are presented as means of three measurements \pm standard deviation (SD)).

Differential scanning calorimetry analysis (DSC)

DSC thermogram of the copolymer, shown in Fig. 4 exhibited a single glass transition temperature (T_g) at 54.6 °C, an endothermic process at 72.8 °C that can be attributed to the evaporation of water or volatile low molecular products and two degradation processes at 99.7 and respectively at 148.8 °C. The first exothermic process could be attributed to some polymerization process (not entirely finished in the synthesis step)

but also to some degradation products, especially water and carbon dioxide, meanwhile second exothermic process (with a weight loss of 16.50 wt.%) could be assigned to the degradation processes induced by the presence of oxygen in the copolymer structure.

The results are in good agreement with the literature, since it has been reported that the copolymers of HEMA exhibited lower glass transition temperatures compared to poly(HEMA)¹³, which T_g was reported at 88.2 °C.¹⁴

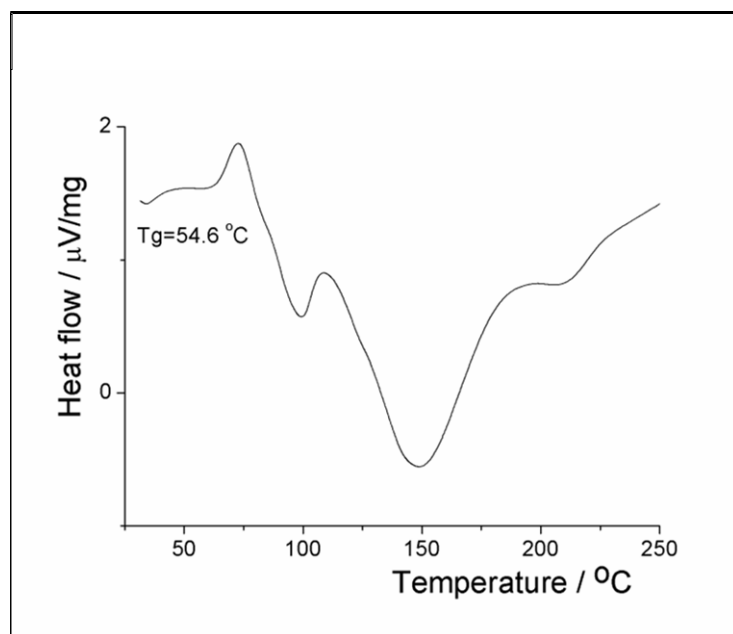


Fig. 4 – DSC thermogram of PHEMA-U.

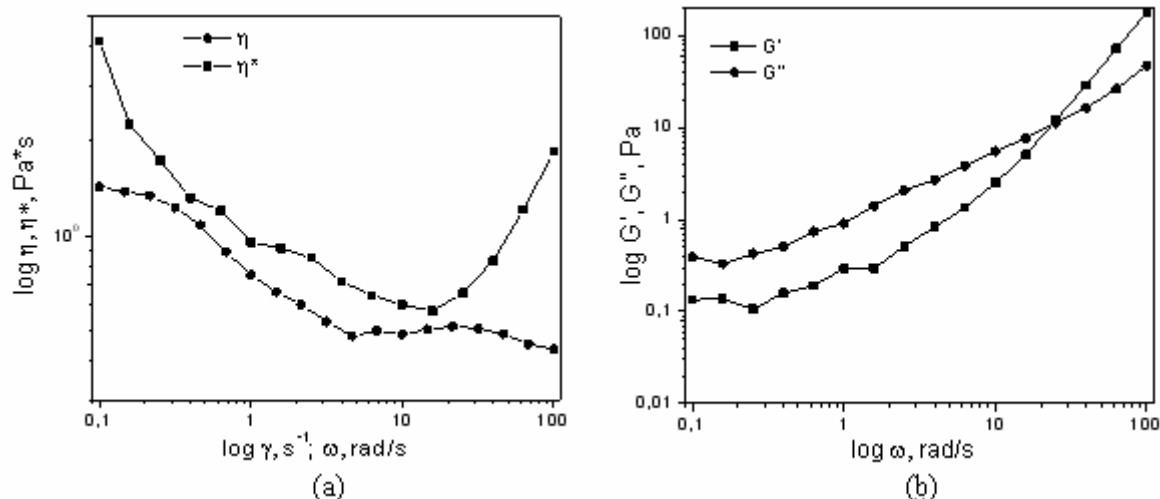


Fig. 5 – Variation of viscosity with shear rate and angular frequency (a) and storage and loss moduli (b) with angular frequency, at constant temperature (25 ± 0.1 °C), for PHEMA-U solutions (20%, w/w).

Rheological studies

The rheological plot of $\log \eta$ (steady shear viscosity), η^* (complex viscosity) vs. $\log \gamma$ (shear rate) and $\log \omega$ (angular frequency) are compared in Fig. 5a. Rotational tests were used to determine the viscosity of the synthesized copolymer solution as a function of shear rate. Also, small amplitude oscillatory shear measurements were performed in the short LVE range (0-100 rad/s). The complex viscosity varied from 4.16 to 1.85 Pa.s while the steady shear viscosity varied from 1.23 to 0.44 Pa.s.

The shape of the viscosity curves clearly shows that at shear rates exceeding 0.1 s^{-1} , the viscosity of the polymer solutions decreases with increasing shear rate and respectively angular frequency, indicating that the PHEMA-U solution behave as non-Newtonian (pseudoplastic), shear-thinning fluid. The shear-thinning viscosity behavior of the copolymer becomes relatively less pronounced as the shear rates exceed 10 s^{-1} – Fig. 5a.

The term zero shear viscosity is the viscosity measured in shear deformation at a shear rate of zero. The zero-shear viscosity (η_0) was determined from the shear data using the Carreau-Yasuda model. The η_0 for PHEMA-U solution was 0.815 Pa.s.

The oscillatory tests were performed mainly to determine the dynamic moduli, i.e., storage (G') and loss moduli (G'') in the linear viscoelastic region. G' is elastic or storage modulus and quantifies the solid-like or structured nature (i.e., energy storage) of a material. The viscous or loss modulus G'' quantifies the liquid-like nature (i.e., viscous dissipation) of a material. The ratio of loss modulus to storage modulus gives the phase angle, $\tan(\delta) = G''/G'$. Thus, G' and G'' provide

information on the microstructure of a polymeric material by decoupling its elastic and viscous properties.^{15,16} Fig. 5(b) illustrates the dynamic moduli G' , G'' as function of frequency for PHEMA-U copolymer solution. At low angular frequencies the G'' value is higher than G' , indicating a liquid-like behavior of the solution, while at higher angular frequencies storage modulus become slightly higher than loss modulus corresponding to a solid-like behavior. The cross-over point of the two moduli appears at $\omega = 25 \text{ s}^{-1}$, $G' = G'' = 11.8 \text{ Pa}$.

Fluorescence spectroscopy

Fluorescence spectroscopy can be used to study molecular interactions and motions: including macromolecular rotational diffusion, solvent reorientation and energy transfer or motions of domains.¹⁷ Fluorescence is a photon emission process that occurs during molecular relaxation from electronic excited states and involves transitions between electronic and vibrational states of polyatomic fluorescent molecules (fluorophores).¹⁸ A fluorophore is a component that absorb energy of a specific wavelength and then re-emit energy at a different but equally specific wavelength.¹⁹

The fluorescence emission spectra of the copolymer are shown in Fig. 6. A strong band was registered at 343 nm that can be attributed to the presence of the hydrophobic spiroacetal moiety, which confers specific conformational configuration to the copolymer. With the increasing of the concentration a higher intensity was recorded. Thus, fluorescence spectroscopy confirmed the structure of the copolymer.

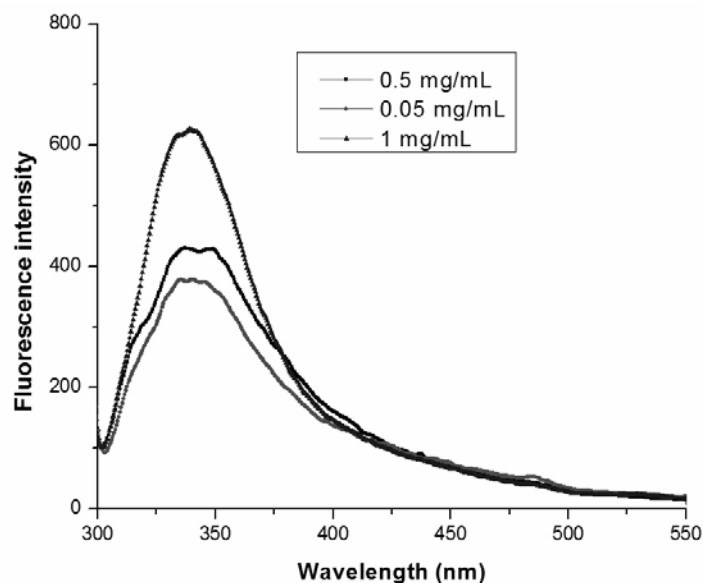


Fig. 6 – The fluorescence spectra of PHEMA_U solutions.

Dielectric spectroscopy analysis

The dielectric properties of PHEMA_U have been evaluated in order to investigate the relaxation processes. An important intrinsic characteristic of a material, expressed by the complex dielectric permittivity might be separated into its real and imaginary components, $\epsilon^* = \epsilon' - i\epsilon''$, where ϵ' is the dielectric constant and ϵ'' is the dielectric loss. The temperature dependence of both, real and imaginary part, are plotted in Fig 7. At low temperatures (Fig. 7a) ϵ' increases slightly and linearly because only a few polarisable units could follow the electric field but at high temperatures, the dipoles absorb enough energy and the magnitude of ϵ' sharply increases.

Also, the dielectric constant decreases gradually with increasing frequency because, at low frequencies, the dipolar moments has enough time to orient themselves in the direction of the external field while, at high frequencies, the dipoles can not follow the alternative field.

Accordingly to Fig. 7b, the evaluated copolymer provided two relaxation processes, namely γ -relaxation and β -relaxation that are initiated by localized motions of polar groups from side chain macromolecules, or parts of them, without disturbing the main chains. According to the literature²⁰, β -relaxation is assigned with the rotation of the ester side group and the γ -relaxation corresponds to the rotation of hydroxymethyl group side.

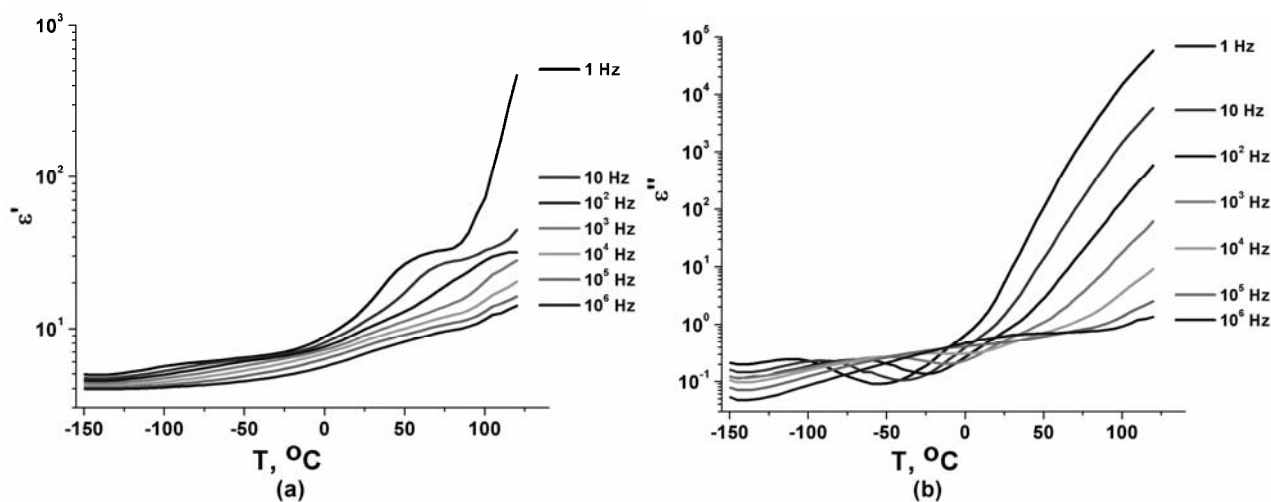


Fig. 7 – Logarithmic plot of the dielectric constant (a) and the dielectric loss (b) as a function of temperature at selected frequencies.

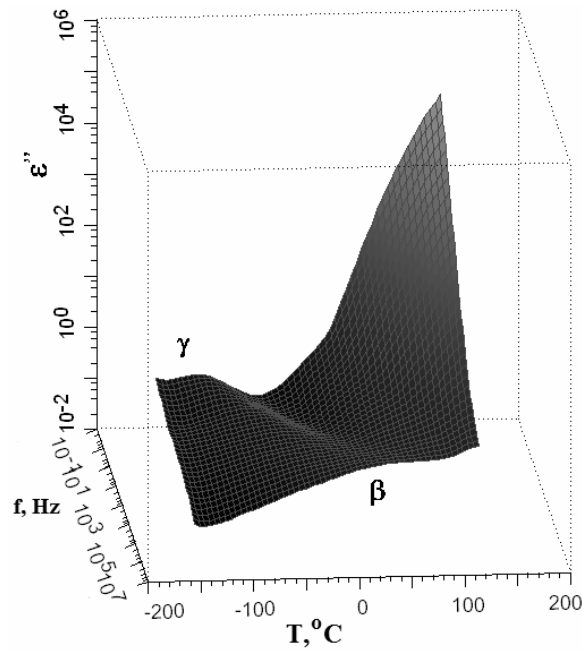


Fig. 8 – The evolution of dielectric loss for PHEMA_U as a function of temperature and frequency.

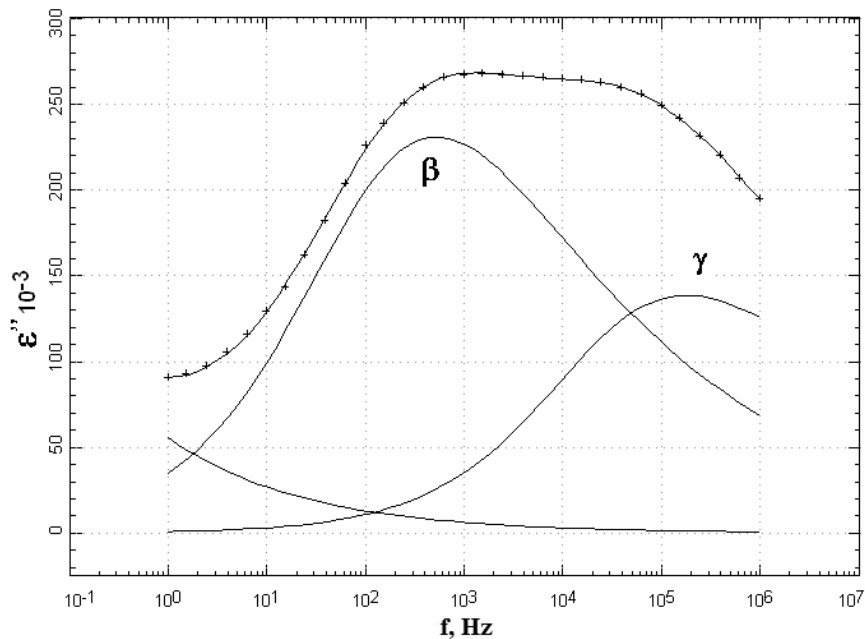


Fig. 9 – The HN fitting technique for both γ and β -relaxation processes at temperature of $-55\text{ }^{\circ}\text{C}$.

The dielectric loss behavior as function of frequency and temperature (Fig. 8) confirm the existence of sub-glass transitions, γ and β -relaxations, respectively. The γ -relaxation transition is observed as a peak at $-120\text{ }^{\circ}\text{C}$ that moves to higher frequencies as temperature increasing up to $-20\text{ }^{\circ}\text{C}$. Furthermore, β -relaxation is clearly recognized as a peak at $-90\text{ }^{\circ}\text{C}$ that shifts to higher frequencies as temperature increasing and ends at $-15\text{ }^{\circ}\text{C}$.

The HN fitting process for all γ - and β -relaxation spectra is illustrated in fig. 9. The fit function consists in a sum of two HN functions, one for γ -relaxation at high frequencies and one for β -relaxation at low frequencies, and supplementary a fit exponential term that takes into consideration the conductivity effects.

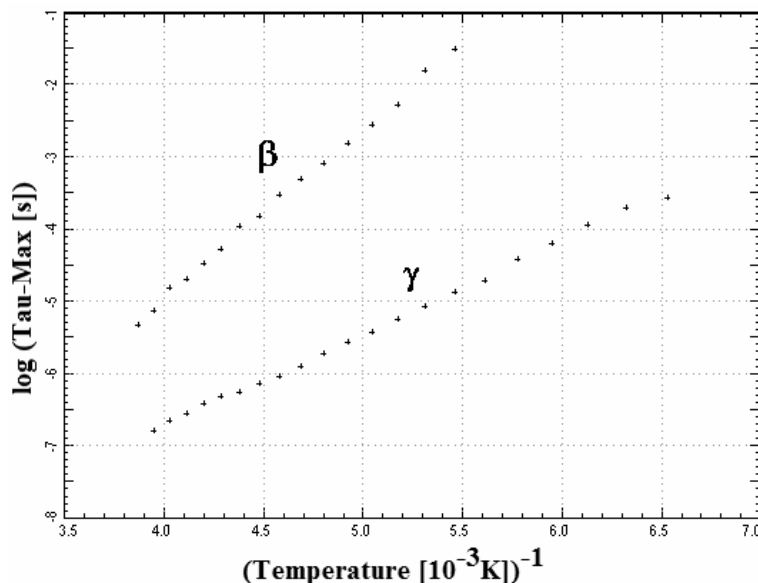


Fig. 10 – Arrhenius plots representation of γ - and β -relaxation processes.

The temperature dependences of the relaxation times are presented in Fig. 10, according to Arrhenius plots. The representation $\log \tau_{\max} = f(1/T)$ allows determining the numerical values for the relaxation type processes. The γ -relaxation process is defined by the relaxation time of 6.221×10^{-12} s and its activation energy of 19.9 kJ mol^{-1} . The β -relaxation process is defined by the relaxation time of 6.047×10^{-14} s and its activation energy that is found to be 36.3 kJ mol^{-1} . Analyzing both γ and β -relaxation processes from Arrhenius plot representation it can be concluded that the activation energy of β -relaxation process is higher than that of γ -relaxation process, meaning that the energy for ester-type groups rotation is higher than that of hydroxymethyl-type sides.

EXPERIMENTAL

Materials

2-hydroxyethyl methacrylate (HEMA) (Fluka, purity > 96%) was purified by passing it through an inhibitor removal column. The inhibitor-remover replacement packing for removing hydroquinone (HQ) and hydroquinone monomethyl ether (MEHQ) was purchased from Aldrich. 3,9-Divinyl-2,4,8,10-tetraoxaspiro[5.5] undecane – (U) (Aldrich, 98%) and 1,4 dioxane were used without further purification. 2,2' azobis 2-methylpropionitrile (AIBN) was purified by recrystallized from methanol. Water used in all experiments was purified using an Ultra Clear TWF UV System.

Polymerization Process

The copolymer PHEMA-U was prepared by radical polymerization process using AIBN as initiator, 1,4-Dioxane as solvent, under nitrogen atmosphere, at 75°C for 17 h. The

reaction was performed in a constant temperature bath, with a stirring rate of 250 rpm. The reaction recipe is presented in Table 2. After synthesis the copolymer was dried in a vacuum oven for 24h.

Molecular weight evaluation. The molecular weight and the second virial coefficient (A_2) of the synthesized copolymer were evaluated using a Zetasizer NanoS system that provides a method for measuring the molecular weight of the polymers at only an angle (173°) by using Rayleigh equation (eq. 1) – a relationship between the intensity of the scattered light of macromolecules and their weight-average molecular weight (Mw). A plot of KC/R_θ versus C is expected to be linear with an intercept equivalent to $1/M$ and a slope equal to the second virial coefficient A_2 . The following relations (Rayleigh equation) are valid:

$$\frac{KC}{R_\theta} = \left(\frac{1}{M} + 2A_2C \right) P(\theta) \quad (1)$$

where

$$K = \frac{2\pi^2}{\lambda_0^4 N_A} \left(n_0 \frac{dn}{dc} \right)^2 \quad (2)$$

$$P_\theta = 1 + \frac{16\pi^2 n^2 R_g^2}{3\lambda^2} \sin^2 \frac{\theta}{2} \quad (3)$$

and

$$R_\theta = \frac{I_A n_0^2}{I_T n_T^2} R_T \quad (4)$$

where: K – optical constant, M – molecular weight, A_2 – 2nd virial coefficient, C – polymer concentration, R_θ – Rayleigh ratio of the sample, $P(\theta)$ – shape factor; n_0 is the refractive index of the solvent, the laser wavelength, N_A – the Avogadro constant, and dn/dc is the refractive index increment of the scattering species in the solvent used. R_g – radius of gyration; θ – measurement angle, I_A – intensity of sample, I_T – intensity of standard (toluene), n_T – standard toluene refractive index, R_T – Rayleigh ratio of standard (toluene).

Table 2

The recipe for the copolymer solution preparation

Components	Quantities (mMols)
2-hydroxyethyl methacrylate (HEMA)	8.48
3, 9-Divinyl-2, 4, 8, 10-tetraoxaspiro[5.5]undecane – (U)	4.25
2,2' azobis 2-methylpropionitrile (AIBN)	1.1
1,4 dioxane	94

Z-average size (d, nm) versus temperature. Hydrodynamic diameter of the copolymer (1% in DMSO) was determined by dynamic light scattering technique using a Zetasizer model Nano ZS device, with red laser 633 nm He/Ne from Malvern Instruments, UK. The experiments were conducted to investigate the dependence of copolymer particles size versus temperature effect, in the range of 22–40 °C, in triplicate.

Differential scanning calorimetry analysis (DSC). Thermal analysis - DSC was performed with an apparatus STA 449F1 Jupiter model (Netzsch –Germany). The sample mass (7 mg) was heated from 25 °C to 250 °C, with the heating rate of 5 °C/min, in Al crucible and Al was used as reference material. The nitrogen (99.99 % purity) was used as carrier gas with flow rate of 50 ml/min and protective purge for thermobalance of 20 mL/min.

Rheological studies. The rheological behavior of the copolymer solution (20%(w/w)) was investigated at constant temperature (25 ± 0.1 °C), with a Physica MCR 301 rheometer (Anton Paar), using a plate-plate geometry of 25 mm as a measurement system. Strain sweep tests were performed at 25 °C at 10 rad/s over the strain range 0.01–100% to determine the linear viscoelastic region (LVE), in which G' and G'' are practically constant and independent of the strain amplitude. Oscillatory shear tests were made in the frequency range 0.1–100 s⁻¹.

Fluorescence spectroscopy. The emission spectra of the copolymer solutions with various concentrations (0.05 mg/mL, 0.5 mg/mL and 1mg/mL) in 1,4-Dioxane were recorded using Perkin Elmer fluorescence spectrophotometer. The samples were excited at 274 nm, and the emission spectra were obtained in the range of 280–550 nm at an integration time of 1.0 s, with the slit width for excitation and emission of 10 and respectively 7 nm.

Dielectric spectroscopy. Complex dielectric permittivity measurements have been performed using the Novocontrol Dielectric Spectrometer (GmbH Germany), CONCEPT 40 in the range of frequency (1 – 106 Hz) and temperature (-150 – 120°C), at the amplitude of applied voltage of 1 V, in nitrogen atmosphere avoiding water absorption. A Novocontrol Quatro Cryosystem device has been used in order to control the temperature with 0.1°C stability. The samples has been prepared as pellets with 0.6 mm thick and then sandwiched between two gold coated plate electrodes.

CONCLUSIONS

A polymeric system based on poly (2-hydroxyethyl methacrylate) and 3, 9-divinyl-2, 4, 8, 10-tetraoxaspiro (5.5) undecane (U) have been characterized with regard to physiochemical properties, such as molecular weight, temperature-

sensitive abilities, thermal stability, rheological and dielectrical properties with the aim to find new usefull insights for future applications. DLS analysis indicated that thermosensitive character of the evaluated compound is mainly attributed to the presence of the spiroacetal moiety. Broadband dielectric spectroscopy analysis provided data about real and imaginary components of the permittivity. The copolymer exhibited two relaxation processes attributed mainly to the non-cooperative motion of side groups only, or parts of them.

Acknowledgements: This work was financially supported by the grant of the Romanian National Authority for Scientific Research, CNCS-UEFISCDI, project number PNII-RU-TE-2014-4-0294 „Novel hydrogels synthesis with defined 3D functionality and biodegradable characteristics for bioapplications”.

REFERENCES

1. “Smart Polymers. Applications” in “Biotechnology and Biomedicine”, I. Galaev and B.Mattiasson (Eds.), CRC Press Taylor & Francis Group, 6000 Broken Sound Parkway NW, Suite 300; Boca Raton, FL, by Taylor & Francis Group, LLC; CRC Press, **2008**.
2. M. D. Blanco, O. Garcia, C. Gomez, R. L. Sastre and J. M. Teijon, *J. Pharm. Pharmacol.*, **2000**, 52, 1319–1325.
3. A. P. Chiriac, L. E. Nita and M. T. Nistor, *J. Polym. Sci. A1*, **2011**, 49, 1543–1551.
4. A. P. Chiriac, L.E. Nita and I. Neamtu, *Mat. Sci. Eng. C*, **2015**, 47, 17–25.
5. A.P. Chiriac, M.T. Nistor, L.E. Nita and I. Neamtu, *Rev. Roum. Chim.*, **2013**, 58, 243–253.
6. C. Billaud, M. Sarakha and M. Bolte, *Eur. Polym. J.*, **2000**, 36, 1401–1408.
7. A. M. Imroz Ali, K. Tauer and M.Sedlak, *Polym.*, **2005**, 46, 1017–1023.
8. L. E. Nita, A. P. Chiriac and M. T. Nistor, *Coll. Surf. A*, **2011**, 381, 111–117.
10. B. A. Kulkarni, G.P. Roth, E. Lobkovsky and J.A. Porco, *J. Comb. Chem.* **2002**, 4, 56–72.
11. S. Nguyen and M. D. Buschmann, *Carbohydr. Polym.*, **2009**, 75, 528–533.
12. A. P. Chiriac, L. E. Nita, N. Tudorachi, I. Neamtu, V. Balan and L. Tartau. *Mat. Sci. Eng. C*, **2015**, 50, 348–357.
13. A.P. Chiriac, L. E. Nita and M. T. Nistor, *J. Nanopart. Res.*, **2011**, 13, 6953–6962.

14. K. Mohomed, F. Moussy, J. P. Harmon. *Polym.*, **2006**, 47, 3856–3865.
15. T. Caykara, C. Ozyurek, O. Kantoglu and B. Erdogan, *Polym. Degr. Stab.*, **2003**, 80, 339-343.
16. D.Filip, D.Macocinschi, E. Paslaru, B. S. Munteanu, R. P. Dumitriu, M. Lungu and C. Vasile. *J Nanopart Res*, **2014**, 16, 2710-2727.
17. M. Brader “Biopharmaceutical Development” in “Biophysical Characterization of Proteins in Developing Biopharmaceuticals”, Elsevier Radarweg 29, PO Box 211, 1000 AE Amsterdam, Netherlands, **2015**, p. 87-107.
18. “Fluorescence Spectrophotometry” in “Encyclopedia of Life Sciences”, Peter TC So, Chen Y DongMacmillan Publishers Ltd, Nature Publishing Group.
19. M. Sauer, J. Hofkens and J. Enderlein, “Handbook of Fluorescence Spectroscopy and Imaging”, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.
20. K. Mohomed, T.G. Gerasimov and F. Moussy, J.P. Harmon. *Polym.*, **2005**, 46, 3847–3855.