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Dedicated to the memory of
Dr. Henry V. Kehiaian (1929–2009)

BIOMATERIALS BASED ON 2-HYDROXYETHYL METHACRYLATE: THE INFLUENCE OF THE INITIATOR TYPE

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The polymerization process of 2-hydroxyethyl methacrylate (HEMA) with an acetal-type crosslinking agent – 3, 9-divinyl-2,4,8,10-tetraoxaspiro[5.5]-undecane (U) – was investigated. The copolymers synthesis has been performed in emulsion in the presence of a water soluble initiator: 4, 4'-azobis(cyanopentanoic acid)(ACPA) and respectively by dispersion using 2,2′-Azobis(2-methylpropionitrile)(AIBN) as initiator. The comonomer presence determined a slowly decrease of the conversion. Chemical structure and composition were evaluated by ^{1}H-NMR spectroscopy. The study evidenced the stability of the spiro-acetal cycle during the polymerization process. The particle size evolution in interdependence with the registered conversion is presented. The synthesis induced by the AIBN presence displays particles much smaller – 120 nm for PHEMA and 100 nm for the copolymer – than using ACPA as initiator, the particles size being in this case of about 170 nm for PHEMA and 240 nm for the copolymer. Also, the presence of the comonomer determined the decreasing of the zeta potential and the conductivity of the particle. The morphological differences between the synthesized homo- and copolymers were proved through AFM investigations.

INTRODUCTION

In the emulsion polymerization any kind of surface-active agents play the crucial role of stabilizing the resulting latexes due to the existence of their specific groups at the interface.[1,2] Such groups can be introduced either by initiator residues, comonomers, but in most of the practical cases by adsorbed surfactants.[1] The properties of the monomer(s) and polymer(s), the initiating species, the surface-active molecule(s), other auxiliary materials and the process variables such as temperature, feeding profiles, and hydrodynamic forces (stirring speed etc.) influence the hydrophilic–hydrophobic conditions in the heterophase polymerizations and the finally colloidal stability. In case of the highly water-soluble monomers initiation takes place in aqua solution where the homogeneous nucleation mechanism dominates, some times a coagulative nucleation mechanism being also possible.[3,4] The interest in the preparation of PHEMA latex is entirely justified the homopolymer being a compatible biomaterial.[5-7] Reports mention the preparation of the polymer by bulk polymerization with low water content or by suspension polymerization to form microbeads.[8,9] PHEMA is usually reported to be biocompatible but not biodegradable.[10] Polymerization of HEMA in aqueous media, where HEMA is not only the monomer but also a co-solvent, posses all features of dispersion polymerization. Thus, the reaction system is homogenous before starting the chain growth and becomes heterogeneous as the polymer precipitates with increasing conversion.[2] The classical emulsion polymerization of HEMA with the goal to prepare PHEMA particles in the nanometer size range faces a lot of problems as the

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size of the polymer particles typically prepared in dispersion polymerizations are in the mm-size range. On the contrary, suspension polymerization is state of the art for the preparation of monodisperse PHEMA particles in the size range of a few hundreds of micrometers for biomedical applications.[11] The literature in the field mentions the use of HEMA as comonomer in emulsion polymerization in order to hydrophilize particle surfaces and to improve latex stability, as well as the preparation of PHEMA particles in a size range of about 100 nm using sodium dodecyl sulfate and 2,2'-azobisisobutyronitrile (AIBN) as stabilizer and initiator, respectively.[3,4,12-16] The influence of the initiators on HEMA polymerization was also presented including the effect of the initiators aqua solubility. The achieved PHEMA nanoparticles were thought useful for various applications or investigations where hydrophilic and biocompatible nanoparticles are required as model investigations of phagocytosis for example replacing the micrometer-sized particles.[1,16]

The present investigation refers to the preparation of the copolymers based on 2-hydroxyethyl methacrylate (HEMA) with an acetal-type crosslinking agent – 3, 9-divinyl-2,4,8,10-tetraoxaspiro[5.5]-undecane (U). The copolymers were achieved by emulsion polymerization in the presence of a water soluble initiator 4, 4'-azobis(cyanopentanoic acid) and by dispersion polymerization using AIBN as initiator. Kinetic aspects – conversion, polymerization rate – were correlated on the particles dimension and the surface particles charge (the zeta potential) and the polymerization technique. The final goal is to obtain copolymeric stable nanoparticles as future matrix for bioactive substances entrapment.

EXPERIMENTAL

Materials

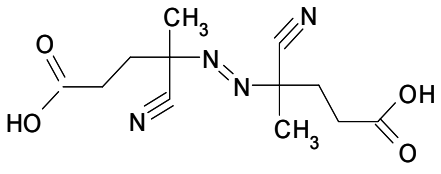
HEMA (from Flucka, purity>96%) was purified by passing it through an inhibitor removal column. The inhibitor-remover replacement packing (for removing HQ and MEHQ) was purchased from Aldrich. 3,9-divinyl-2,4,8,10-tetraoxaspiro[5.5]undecane – U (Aldrich, 98%), sodium lauryl sulfate ($C_{12}H_{25}O_4SNa$) – (SLS) from Sigma (c > 95 wt %), poly(vinyl alcohol) – PVA from Oriental Chemical Industry (Mw=120000 Da, degree of hydrolysis = 88) and 4,4'-azobis(cyanopentanoic acid) (ACPA) (Fluka, 98%), 2, 2'-azoisobutyronitrile (AIBN) (Fluka 98%) were used without further purification. In all experiments it was used twice distilled water which contained no foreign ions.

Polymerization Process

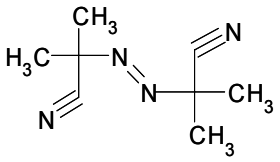
Polymerization recipes are presented in Table 1. Polymerizations were conducted at 80 °C, in 100 cm^3 four-necked round-bottomed flasks in a constant temperature bath, with a mechanical stirring rate of 180 rpm.

Characterization

The structural analysis of the samples has been performed by ^{1}H-NMR (Fig. 1).



4,4-azobis(cyanopentanoic acid)



2, 2-azoisobutyronitrile

Initiators chemical structure

Table 1

	HEMA, M	U, M	ACPA or AIBN, M	SLS, M	PVA, g	Water, g
PHEMA	0.03076	-	5×10^{-4}	9.26×10^{-4}	0.267	100
P(HEMA-co-U): 98/2	0.03015	3.77×10^{-4}	5×10^{-4}	9.26×10^{-4}	0.267	100

Constants of decomposition rate of initiators:
K_d (s^{-1}) of 4,4-Azobis(4-cyanovaleric acid)(ABCPA; M_w = 280.28) at 70°C = 1.9×10^{-5}
K_d (s^{-1}) of 2,2'-Azobisisobutyronitrile (AIBN; M_w = 164.21) at 70°C = 3.2×10^{-5}

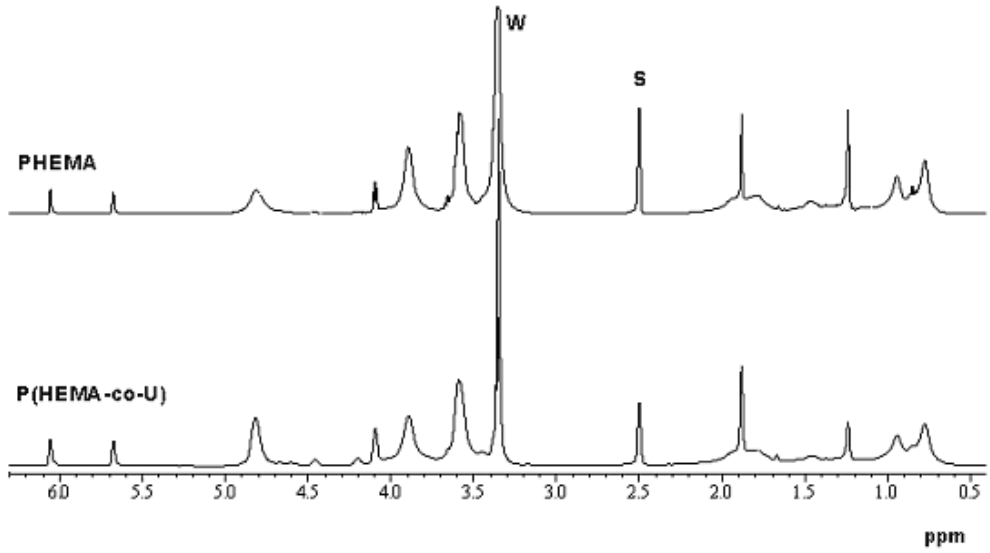


Fig. 1 – The ^{1}H–NMR spectra of the copolymers comparative with PHEMA.

The ^{1}H-NMR tests were performed with a Bruker Avance DRX 400 spectrometer equipped with a 5 mm broad band probe. ^{1}H-NMR spectra were recorded at a frequency of 400 MHz.

The spectrum confirms the differences between the polymeric structures P(HEMA) and P(HEMA-co-U) through the appearance or modification of signals at 3.5, 4.2, 4.5, 4.9 ppm, which are characteristic for the U–comonomer. Also, the attenuation of the signals at 1.8 ppm indicates that the majority of the end double bonds of the monomers have been converted into single bonds to form the main chain during copolymerization.

The conversion was gravimetrically determined. The polymerization rate was determined from the slope of the conversion – time curve in the constant rate region.

AFM surface analysis

AFM measurements are performed in air at room temperature, in the tapping mode using a Scanning Probe Microscope (Solver PRO-M, NT-MDT, Russia) with commercially available NSG10/Au Silicon cantilevers. The manufacturer's values for the probe tip radius are 10 nm, and the typical force constant is 11.5 N/m. In the tapping mode, the cantilever is oscillated at a frequency of 254.244 kHz. Representative scans of the film surface (scan physical size is 50 μm x 50 μm) are obtained for each sample. The Root Mean Square (RMS) roughness parameter, Sq, which is the root mean square of the surface departures from the mean plane within the sampling area, is extracted from the Nova software provided (Eq. 1):

$$S_q = \sqrt{\frac{1}{MN}\sum_{j=1}^{N}\sum_{i=1}^{M} z^2(x_i y_j)} \qquad (1)$$

where M is the number of columns in the surface and N is the number of rows in the surface. Off-line image analysis on quantification of surface properties using the surface roughness parameter is obtained.

The particle size distribution was determined by using a dynamic light scattering technique (Zetasizer model Nano ZS (Malvern Instruments, UK)) with red laser 633 nm (He/ Ne). The system uses non-invasive back scatter (NIBS) technology wherein the optics are not in contact with the sample, back scattered light being detected. The use of NIBS technology reduces multiple scattering effects and consequently size distributions in higher concentrations of sample can be measured. This is the system for which the Mie method is applied over the whole measuring range from 0.6 nm to 6 μm.

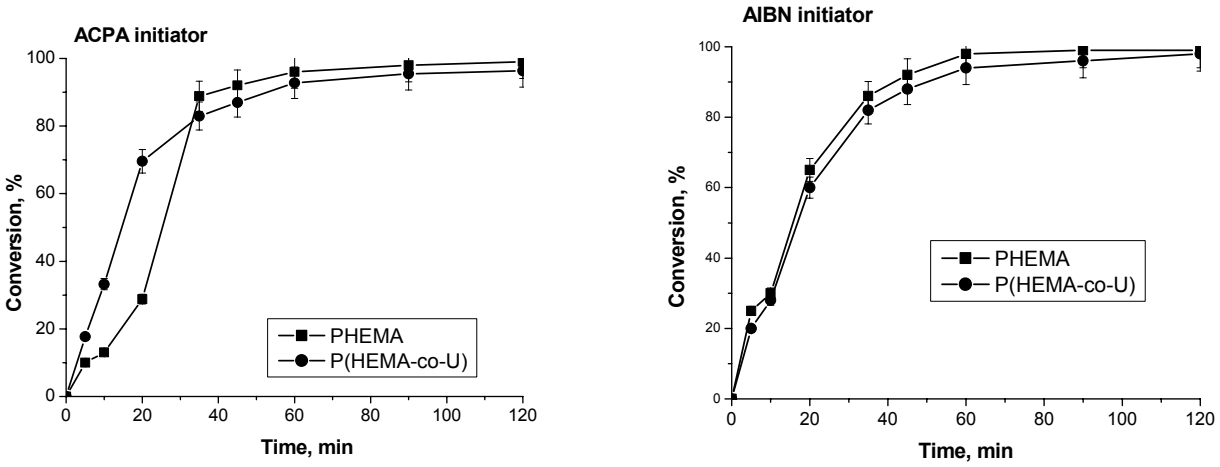
The Zeta potential (ξ) was estimated with the same equipment (Zetasizer model Nano ZS; Malvern Instruments, UK) and it was calculated from the electrophoretic mobility (μ) using the Smoluchowski relationship:

$$\xi = \eta\mu/\varepsilon \text{ with the condition } k\alpha >> 1$$

where η – viscosity, ε – dielectric constant of the medium, k, α – Debye–Huckel parameter and particle radius respectively. The average of five measurements is presented as the mean value for the zeta potential. Also, the difference between the measurements and their average is less than 2.5%.

RESULTS AND DISCUSSION

Fig. 2 illustrates the time-conversion evolution for both ways of the copolymer synthesis in emulsion and dispersion respectively. The results agree well with other data from the literature in case of HEMA emulsion polymerization using SLS and low molecular weight fatty alcohols (or hydrocarbons).[4] Also, the presence of the comonomer (U) determines a slowly decrease of the conversion.



a b

Fig. 2 – Conversion of the samples in the presence of the ACPA (a) and AIBN (b) as initiator.

It is convenient to divide the process of an emulsion polymerization into three distinct steps. Step I is the initial stage where particle formation takes place. Step II is characterized by a constancy of particle number, while polymerization in the particles proceeds in the presence of a separate monomer phase. The beginning of step II is sometimes taken as the conversion where the surfactant concentration drops below its critical micelle concentration (cmc). Step III begins with the disappearance of the monomer droplets, after which the monomer concentration in the particles starts to decrease continuously.[17]

The changes in topography of the copolymer comparative with PHEMA were investigated by atomic force microscopy (AFM) (Fig. 3). The difference between homopolymer (PHEMA) and copolymers morphology was confirmed by AFM measurements. The surface of PHEMA is rather smooth compared to the surface of the copolymer which shows a more granular structure. PHEMA exhibits irregularities ranging from 20 to 80 nm, while the copolymer presents irregularities ranging from 20 to 120 nm. The parameters of root-mean-square (*S*q, giving the standard deviation of the height values), roughness average (*R*a) were obtained from AFM software analyses over a scope of 4 x4 μm (Fig. 3c and 3f). For the homopolymer film, the *R*a and *S*q values are 7.0 nm and 9.5 nm, respectively. The surface of the copolymer film is rougher, the *R*a and *S*q value ranging at 18 nm and 13.5 nm respectively.

Although the nucleation period is quite short, generation of particle nuclei during the early stage of the polymerization plays a crucial role in determining the final latex particle size and particle size distribution and it has also a significant influence on the quality of latex products.[18] The size of the latex particles in emulsion polymerization is also influenced by a number of other factors, including emulsifier concentration, type of initiators, type and stirring rate, and polymerization temperature.[19, 20] At the beginning of the copolymerization process (Fig. 4), the droplets are about 200 nm in diameter, much smaller than the monomer droplets (~ 1000 nm) in conventional emulsion polymerization. After approximately 150 min – in case of using ACPA as initiator – and respectively 100 min – in case of using AIBN as initiator – from the beginning of the reaction the average particle diameter of the homopolymer as well as of the copolymer reaches stable values and maintain this dimension till the end of the polymerization process. Must be also underlined that AIBN as initiator induces the formation of much smaller particles – 120 nm for PHEMA and 100 nm for the copolymer – than using ACPA as initiator – the particles final dimension in this case being about 240 nm for the copolymer and about 170 nm for PHEMA.

The term polydispersity has multiple meanings that are dependent upon the context of its use. In the area of polymer chemistry, polydispersity is defined as the weight average divided by the number average molecular weight (Mw/Mn), and is used to give the researcher an idea of the breadth or width of the molecular weight distribution. In a similar but not identical sense, polydispersity in the area of light scattering is used to describe the width of the particle size distribution. Values greater than 0.7 for PDI indicate that the sample has a very broad size distribution. The maximum value is

arbitrarily limited to 1.0. In Fig. 4 the PDI variation as function of time is illustrated. At the beginning of polymerization process the PDI is higher no matter the initiator type. Then, after 60 minutes in case of the polymerization performed in the presence of AIBN (Fig. 5b) and after 80 minutes when ACPA is used as initiator (Fig. 5a), the PDI decreases. Also, the comonomer addition (U) determines the increase of the PDI especially in the first 100 minutes of the beginning of the reaction. Then, the PDI is maintained in constant limits.

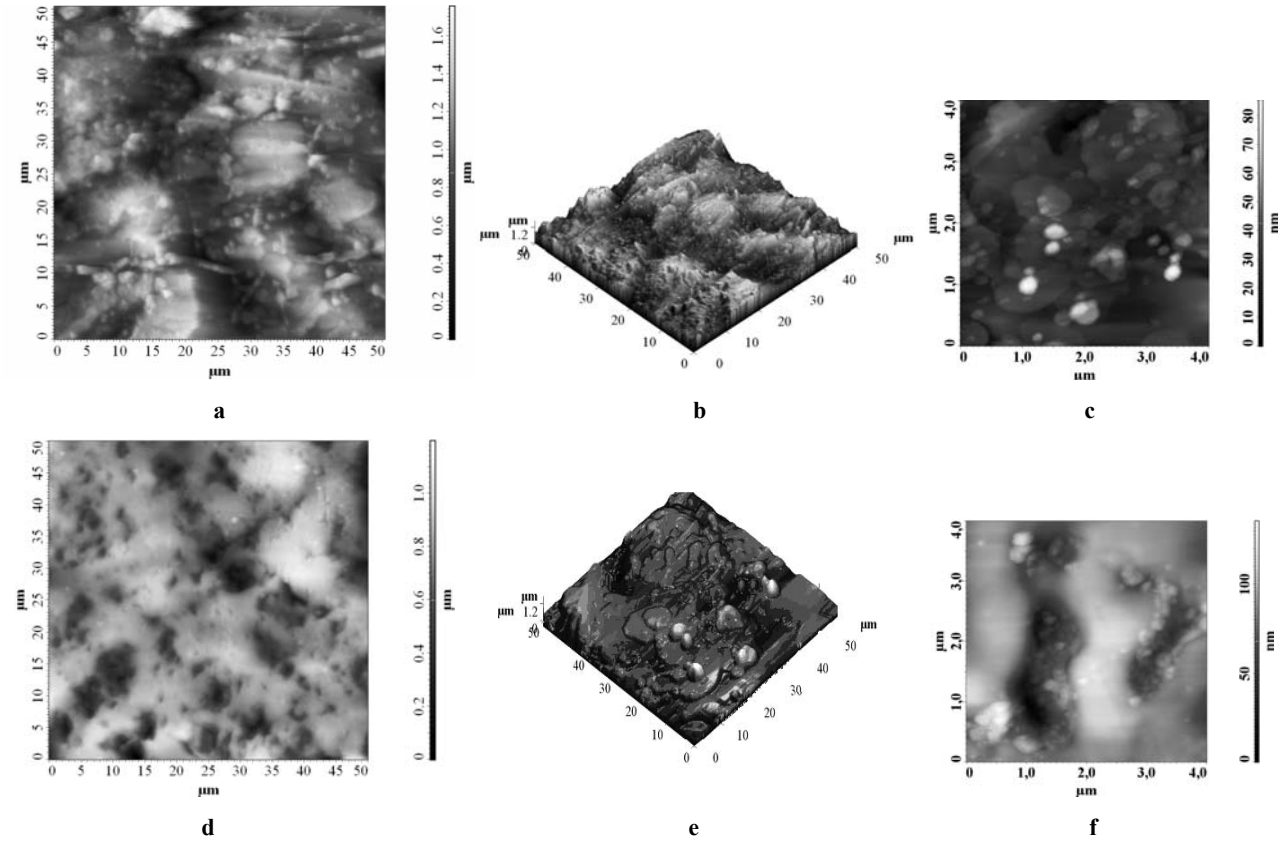
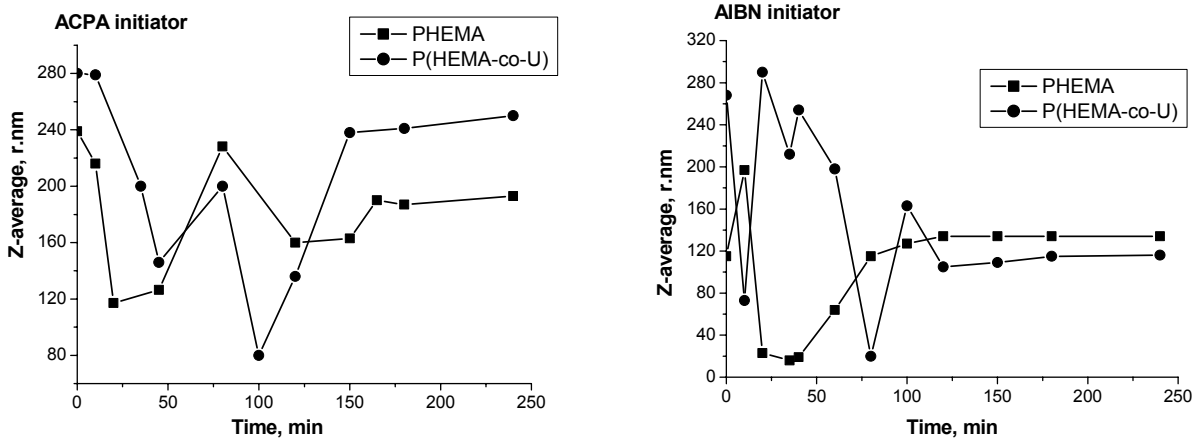
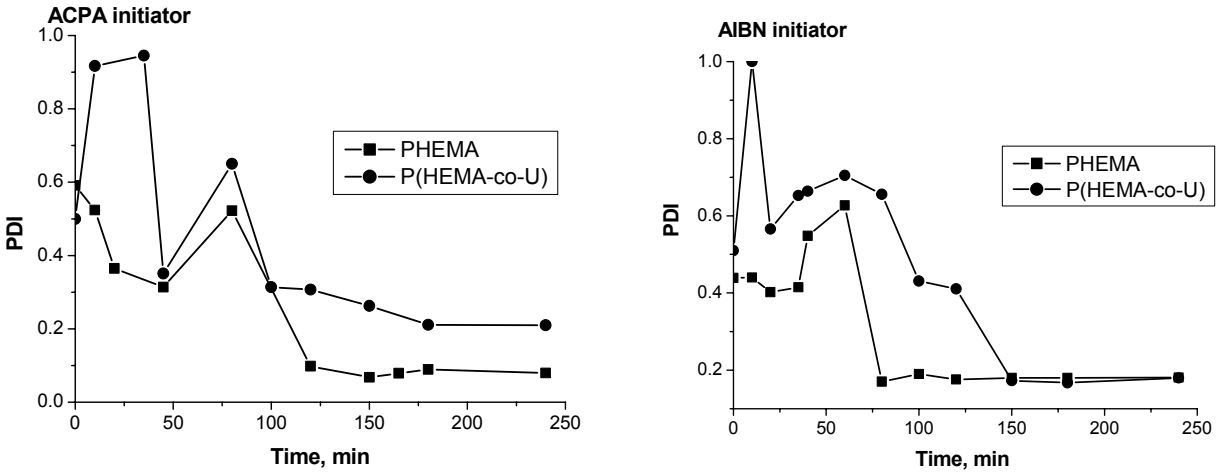


Fig. 3 – The AFM images of **P(HEMA)**: (a) – 2D, (b) – 3D and (c) – 4x4μ detail and for **P(HEMA-co-U)**: (d) – 2D, (e) – 3D and (f) – 4x4μ detail.



a) b)

Fig. 4 – Z-average of the polymeric particles synthesized in the presence of the ACPA (a) and AIBN (b) as initiator.

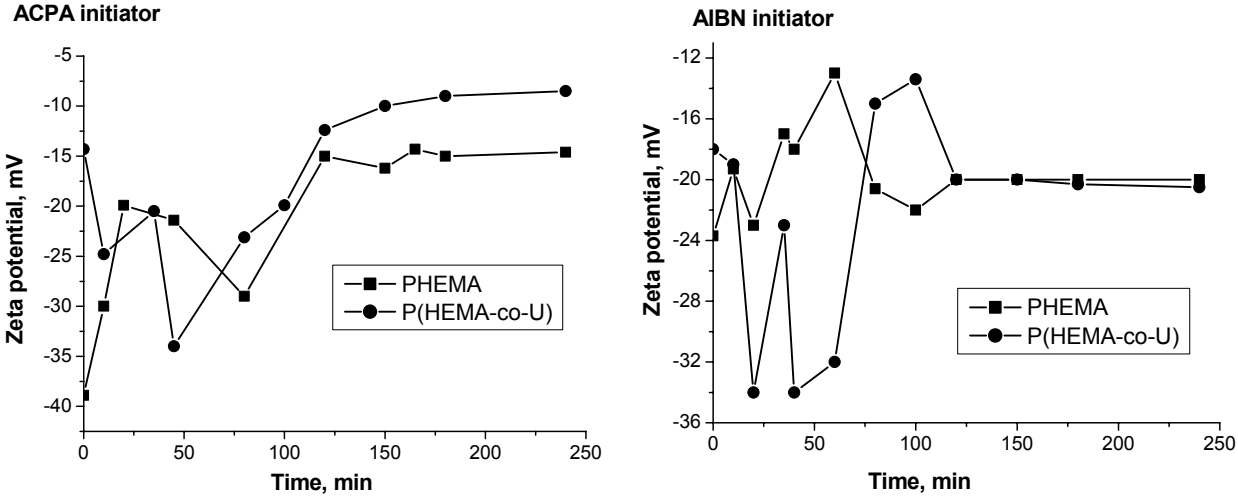


a) b)

Fig. 5 – PDI of the samples obtained in the presence of the ACPA (a) and AIBN (b) as initiator.

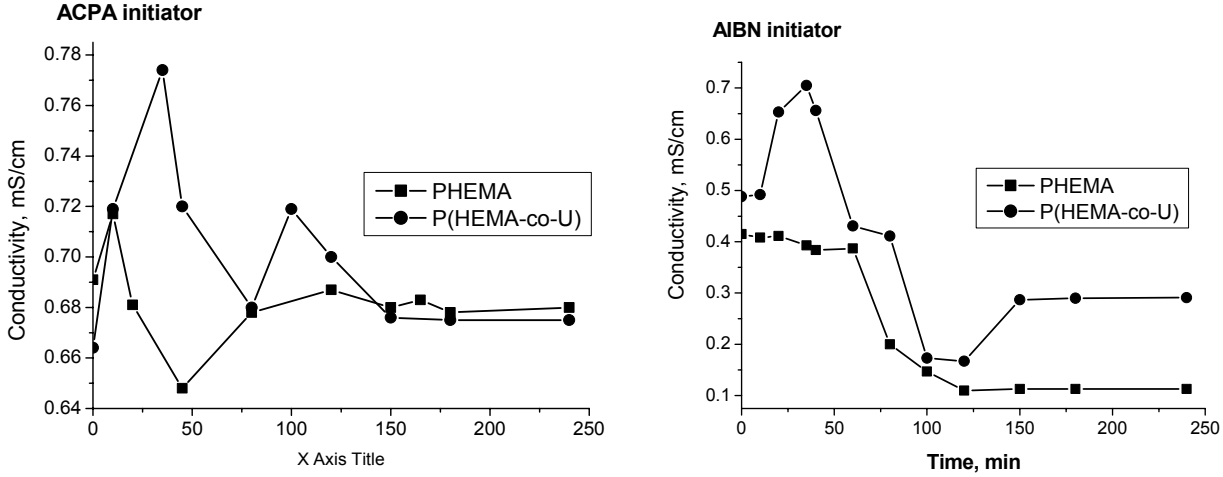
With the zeta potential determination can be obtain data concerning electrokinetic potential in colloidal systems and thus can be established the stability of the colloidal dispersion. The zeta potential indicates the degree of repulsion between similarly charged particles in dispersion and the resistance to aggregation. A comparison between the zeta potential values of the polymeric structures is illustrated in Fig. 6a for samples synthesized in the presence of ACPA as initiator and in Fig. 6b for compounds prepared with AIBN as initiator. An incipient instability corresponds to all variants of synthesized dispersion. Also, the stability of the dispersion is decreased with the increase of the comonomer content (U).

The comonomer addition also determines the increase of the conductivity (Figs. 7 a, b). The particles conductivity values are maintained constant after 120 minutes from the beginning of the reaction. The increase is attributed to the network formation with loosely packed macromolecular chains which allows for a better movement of the functional groups.



a) b)

Fig. 6 – Zeta potential of the samples synthesized in the presence of the ACPA (a) and AIBN (b) as initiator.



a) b)

Fig. 7– Conductivity of particles synthesized in the presence of the ACPA (a) and AIBN (b) as initiator.

CONCLUSIONS

The study presents the preparation of the copolymers based 2-hydroxyethyl methacrylate with an acetal-type crosslinking agent namely 3, 9-divinyl-2,4,8,10-tetraoxaspiro[5.5]-undecane. The copolymers were radicalic synthesized using two initiator type 4, 4'-azobis(cyanopentanoic acid) and 2, 2'-azoisobutyronitrile. The chemical composition was confirmed by ^{1}H-NMR spectroscopy. It was evidenced that the tetraoxaspiro cycle of the comonomer was not opened during the polymerization process. The morphological differences between the homopolymer (PHEMA) and the copolymers were confirmed by AFM measurements. The PHEMA surface is rather smooth compared to the copolymers surface which shows a more granular structure. It was also found that the presence of the crosslinking agent determined a slowly decrease of the conversion. The particle size, zeta potential and conductivity evolution during polymerization process evidenced the influence of the comonomer upon the characteristics of the synthesized polymeric particles.

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