In vitro Release of Metoprolol Tartrate from Poly(vinyl Alcohol)/ Phosphoester – chondroitin Sulfate Semi-IPNs

TACHITA VLAD-BUBULAC*, ANA - MARIA OPREA, DIANA SERBEZEANU, IONELA - DANIELA CARJA, CORNELIU HAMCIUC
"Petru Poni" Institute of Macromolecular Chemistry, 41A Aleea Gr. Ghica Voda, 700487, Iasi, Romania

The present work focuses on the analysis of some semi-interpenetrating polymer networks (semi-IPNs), based on phosphorylated poly(vinyl alcohol) (PVA) and chondroitin sulfat (CS), as sustained release carriers. The investigations were performed on poly(vinyl alcohol)/phosphoester – chondroitin sulfate semi-IPNs hydrogels (PVA/P–CS semi-IPNs) in various mixing ratios. Swelling and drug delivery studies were conducted in a phosphate buffer solution (pH = 7.4) that mimics the pH of the intestinal fluid, at 37 °C. The hydrogels were evaluated for the release of metoprolol tartrate. The release profiles of the drug from PVA/P–CS semi-IPNs were strictly dependent on the CS content: an increase in the percent of released drug was observed with increasing the CS content.

Keywords: phosphorylated poly(vinyl alcohol), chondroitin sulfate, metoprolol tartrate, release kinetics

Biodegradable polymers have attracted considerable attention in the very recent years due to their potential applications in biomedical and ecological fields. Therefore, research efforts are underway to develop new polymeric structures with a biodegradable backbone, having excellent combined features such as biocompatibility, antifouling properties, tunable mechanical properties, no toxicity, potential functional versatility which allows introduction of bioactive molecules and extensive modification of the physical, chemical and biological properties etc. [1–3].

Three dimensional hydrophilic polymer networks, widely known as hydrogels, can absorb and retain a significant amount of water or biological fluids within their structures. The networks are insoluble in water due to the presence of chemical or physical cross-linking which permit hydrogels to be thermodynamically compatible with water [4]. Unlike the other synthetic materials, hydrogels resemble nature living tissue closely in their physical properties due to their water contents and softness. In the biomedical field, hydrogels are used in diagnostic, therapeutic and implantable devices such as catheters, carrier for drug delivery systems, artificially dressing burns, biosensors, cell encapsulation, contact lenses and scaffold for tissue engineering [4,5]. Actually, the development of novel, stable and economical advanced drug delivery formulations based on hydrogels, which are well known to reduce the problems of conventional dosage forms, is an important topic in the field of biomaterials science [6].

Poly (vinyl alcohol) (PVA) is a polymer of great interest because of its many desirable characteristics specifically for various pharmaceutical and biomedical applications [7]. In order to be useful for a wide variety of medicine and pharmaceutical sciences, PVA must be chemically or physically cross-linked. Several difunctional cross-linking agents were used in order to prepare PVA hydrogels: glutaraldehyde, acetaldehyde, formaldehyde etc. But, for pharmaceutical applications, especially when PVA is used as a carrier in drug delivery, the residual crosslinking agent, eventually present in the final hydrogel, could alter the biological activity or degrade the biologically active agent being released. To overcome such inconveniences other less toxic cross-linking agents should be developed.

Polyphosphoesters (PPEs) represent a wide range of biodegradable polymers with repeating phosphoester linkages in the backbone [8]. The pentavalency of the phosphorus atom allows the introduction of bioactive molecules and extensive modification of the physical and chemical properties of the polymers [8, 9]. PPEs are known to be biodegradable [10] and biocompatible and they were widely used in drug [11, 12], protein [9] or gene delivery [13, 14] and tissue engineering [15].

Chondroitin sulfate (CS) is a polysaccharide present in the extracellular matrix of cartilage and tissue in the body serving both important structural and biological functions. CS provides compressive strength to connective tissues by regulating their water content, and possesses characteristic features, such as a high water absorption multifunctionality and biodegradability suitable for bio-applications [2, 16–18].

Metoprolol tartrate is a highly soluble drug which is often used in the treatment of angina pectoris, heart pain, abnormal rhythms of the heart and hypertension [19]. Pharmacokinetics investigations revealed that this drug has a relatively short plasma half-life (3-4 h) while its absorption through the gastrointestinal tract is rapid [20], thus, making metoprolol tartrate a suitable candidate for development of new controlled release formulations.

Actually, chemical crosslinking is a highly versatile method to create or modify polymers with improved properties such as mechanical, thermal and chemical stability. On the other hand, polymer blending is a cheaper and less-time consuming strategy to develop new polymeric materials which combine the properties of more than one existing polymer. Usually, polymer blends are produced by physical mixing of two or more starting polymers and a wide range of final material properties can be designed by appropriate change of material composition. Our interest in the field was previously attributed to the chemical modification of PVA by cross-linking it with aromatic or ciclo-aliphatic phosphonic dichlorides and the blending of PVA/P compositions with CS [21]. The well known bio-tolerance of all the components of PVA/P–CS semi-IPNs structures and their

* *email: tvladb@icmpp.ro, Tel.: +40-232-217454*

behaviour such as porosity, wettability, high swelling ratio inspired us to investigate such formulations for applications in the biomedical field.

Experimental part

Materials

Poly(vinyl alcohol) (PVA) (Mw=18300Da) was purchased from SC ROMCRIL Rasnov; epichlorohydrin was provided by Sigma-Aldrich; chondroitin sulfate (CS) powder, (Mw = 35 kDa) was obtained from Roth (Germany); metoprolol tartrate salt was supplied by Fluka. *p*-Methyl-phenyl phosphonic dichloride was synthesized from *p*-methyl-dichlorophosphine and purified by distillation under reduced pressure according to the method reported in the literature [22]. Phosphate buffer solution (PBS) was prepared from phosphate buffered saline by dissolution in distilled water. All other reagents were used as received from commercial sources or were purified by standard methods.

The PVA/P network has been prepared by nucleophilic displacement reaction of *p*-methyl-phenyl phosphonic dichloride with the hydroxyl groups of PVA according to a method reported previously [23] using dimethylformamide as reaction medium (fig. 1). The experimental details are described in our previous paper [21]. FT-IR (KBr, cm^{-1}): 3390 (O–H stretching), 2960, 2931, 2922 (CH, CH_2 and CH_3 stretching), 1733 (residual acetate CH_3COO^-, carbonyl stretching) and 1430 (C–H bending), 1262 (P=O stretching), 1470 (P–Ar stretching), 969 and 1180, 1150 (P–O–alkyl stretching).

The PVA/P–CS semi-IPNs have been prepared by chemical cross-linking of different weight ratios of PVA/P and CS, in presence of epichlorohydrin under vigorous stirring, according to a published procedure [21]. The obtained gels-like samples were placed on glass plates and then heated at 80 °C for 8 h to afford the completion of the cross-linking process. Afterwards, the hydrogels were extensively washed with water to remove the unreacted compounds and dried for 10 h by using a LABCONCO FreeZone device.

Measurements

The infrared spectra were performed on FT-IR Bruker Vertex 70 Spectrophotometer (Bruker, Germany) either in transmission mode, at frequencies ranging from 400 to 4000 cm^{-1} (with a resolution of 2 cm^{-1} and accumulation of 32 scans), or in reflexion mode, in the 600–4000 cm^{-1} spectral range (64 scans, at 2 cm^{-1} resolution). A Golden Gate ATR accessory (Specac Ltd.) was used in the latter case. The single reflection IRE was diamond, with an incidence angle of 45°.

Scanning electron microscopy (SEM) was performed on a TESLA BS 301 instrument, at 25 kV, with a magnification of 380–3600. The images were recorded on film surfaces deposed on Al supports and coated by sputtering with Au thin films using an EK 3135 EMITECH device.

Swelling studies were performed for all formulations and carried out by direct immersion in PBS (*p*H = 7.4). The samples were maintained for 24 h at 37 °C, periodically taken out from the solution, gently wiped with a soft tissue to remove surface solution, weighed and then placed back into the vessel as quickly as possible. The swelling degree at equilibrium was calculated with the equation 1:

$$Q_{max}(\%) = \frac{W_t - W_d}{W_d} 100 \quad (1)$$

where W_t is the weight of the swollen samples at time t and W_d is the weight of the dry sample.

The kinetics of solvent diffusion into the matrices was determined with the equation 2 [26]:

$$F_t = \frac{W_t}{W_{eq}} = k_{sw} t^{n_{sw}} \quad (2)$$

where W_t and W_{eq} are the amount of 7.4 PBS solution, absorbed by the matrices at time t and at equilibrium, respectively, k_{sw} is the swelling constant characteristic of the system and n_{sw} is the power law diffusion exponent, parameter that dependents on the type of solvent transport.

In vitro drug release studies

The drug loading method was performed by soaking or equilibration of superporous semi-IPN samples in 7.4 PBS solution with metoprolol tartrate for their complete swelling. So, the hydrogels were placed in the drug solution (0.13. %w/v in buffer) and left until all it was sucked up. The swollen hydrogels samples loaded with metoprolol tartrate were dried in an oven at 30 °C overnight.

Drug release

The dissolution medium was *p*H 7.4 PBS solution. During dissolution testing, the media was maintained at 37 ± 0.5 °C. Aliquots of the medium of 1 mL were withdrawn periodically at predetermined time intervals and analyzed at λ_{max} value of 221 nm using a HP 8450A UV–visible spectrophotometer. In order to maintain the solution concentration the sample is reintroduced in the circuit after analyzing.

The concentrations of the drug were calculated based on calibration curves determined for drug at specific maximum absorption wavelengths.

A simple, semi-empirical equation using Korsmeyer and Peppas model was used to kinetically analyze the data regarding the drug release from studied matrices system which is applied at the initial stages (approximately 60 % fractional release) [27]:

$$\frac{M_t}{M_\infty} = k_r t^{n_r} \quad (3)$$

where M_t / M_∞ is the fraction of the drug released at time t, M_t and M_∞ are the absolute cumulative amount of drug released at time t and at infinite time (in this case maximum release amount in the experimental conditions used, at the plateau of the release curves), respectively, k_r is a constant incorporating characteristics of the macromolecular matrix and the drug and n_r is the diffusion exponent, which is indicative of the release mechanism. When n_r is equal or less than 0.5, the release of the drug

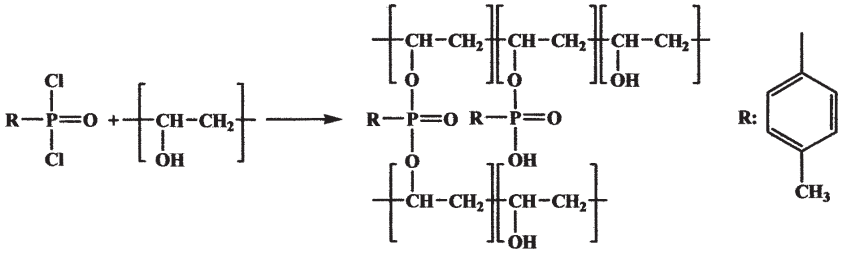

Fig. 1. Preparation of PVA/P networks

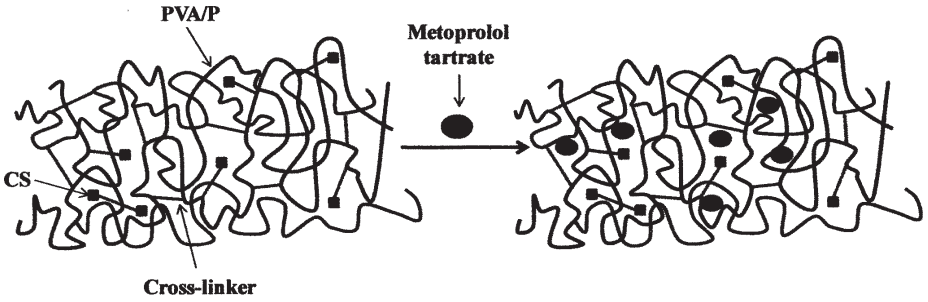

Fig. 2. Schematic representation of PVA/P–CS semi-IPNs loaded with metoprolol tartrate

from the matrix follows a Fickian diffusion mechanism, while a value $0.5 < n_r < 1$ indicates an anomalous or a non-Fickian behaviour. When $n_r = 1$, a case II transport mechanism is involved, while $n_r > 1$ indicates a special case II transport mechanism [28,29]. The corresponding drug-release profiles were represented through plots of the cumulative percentage of drug release versus time.

Results and discussions

The semi-IPN hydrogels based on PVA/P and CS obtained by various mixing ratios of the components (90/10, 80/20, 70/30, 60/40, 50/50) (w/w) were properly coded as 90/10, 80/20, 70/30, 60/40, 50/50. The drug loading method was performed by soaking or equilibration of superporous hydrogels samples in PBS solution (*p*H = 7.4) with metoprolol tartrate for their complete swelling. The swollen hydrogels samples loaded with metoprolol tartrate were dried in an oven at 30°C overnight. A schematic representation of the hybrid PVA/P–CS network loaded with metoprolol tartrate drug is presented in the figure 2. The CS was linked to the PVA/P chains through epichlorohydrin. The PVA/P–CS semi-IPN hydrogels with various mixing ratios were extensively washed with warm water in order to remove the unreacted compounds, especially epichlorohydrin traces, according to a procedure developed by us [21].

FT-IR spectroscopy was used to demonstrate the incorporation of metoprolol tartrate into the PVA/P–CS matrix. Figure 3 shows the FT-IR spectra of 80/20 PVA/P–CS composition, metoprolol tartrate and of the corresponding drug loaded semi-IPN. The loading of the metoprolol tartrate was evidenced by the presence in the FT-IR spectrum (fig. 3b) of the bands located at: 3459 cm^{-1} characteristic for N–H stretching vibration, 2982 and 2876 cm^{-1} characteristic for C–H stretching vibration, 1595 and 1515 cm^{-1} characteristic for COO^- asymmetric stretching and COO^- symmetric stretching vibration, respectively. Other characteristic absorption bands for PVA/P–CS semi-IPNs loaded with metoprolol tartrate were located at 1250 cm^{-1} due to O–H deformation vibration, at 1115 cm^{-1} due to C–O symmetric stretching vibration and at 820 cm^{-1} due to the out of plane O–H vibration of carbonyl groups [30]. FT-IR spectra (fig. 3) evidenced the presence of hydrogen bonds and additional bands characteristic of metoprolol tartrate, showing the interactions between active principle and the hydrogel.

The chemical composition of the semi-IPN hydrogel components affects the swelling ratio of the matrices, the absorption of solvent from the environment that influences the dimensions of the pores and physico-chemical properties of the system. The time to reach maximum swelling degree is about 14 min for 90/10 PVA/P–CS composition and under 10 min for 50/50 PVA/P–CS composition. The swelling degree of the PVA/P–CS semi-IPN hydrogels increases with the amount of CS from 936 % for 90/10 PVA/P–CS to 1836 % for 60/40 PVA/P–CS and of 2254 % for 50/50 PVA/P–CS (fig. 4). The high water quantity uptake and faster swelling can be attributed to the existence of groups with negative charges in CS structure, COO^- and SO^{3-}, which help the gels to swell highly, conferring a high concentration of negative charge in the regions that contain them. The presence of ionization groups on CS chain causes the strong repulsion of negative charges and polar groups so the swelling of the hydrogels is much pronounced than that of nonelectrolyte hydrogels [31, 32]. Furthermore, the high water contents of the hydrogels will likely result in highly macroporous sponge like scaffolds upon lyophilization. All the hydrogel samples exhibited porous structures, the pores size were strictly dependent on the CS content of the hydrogels. Therefore, the solvent molecules could easily diffuse into hydrogels, leading to higher swelling ratio. The values obtained for swelling parameters, n_{sw} varies in range between 0.01–0.17 indicating an anomalous swelling mechanism. The kinetic rate constant, k_{sw} increases with CS content in PVA/P–CS semi-IPN hydrogels from 0.64 $min^{-0.14}$ for 90/10 composition to 0.98 $min^{-0.17}$ for 50/50 composition [21].

The release profiles of metoprolol tartrate from PVA/P–CS semi-IPNs are shown in figure 5. The *in vitro* release of

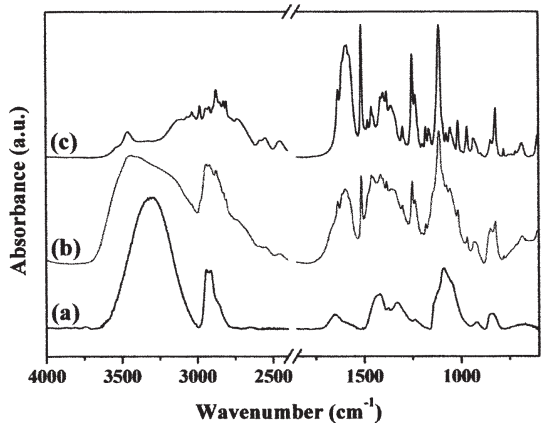

Fig. 3. FT-IR spectra of (a) PVA/P–CS semi-IPN; (b) PVA/P–CS semi-IPN with metoprolol tartrate and (c) metoprolol tartrate

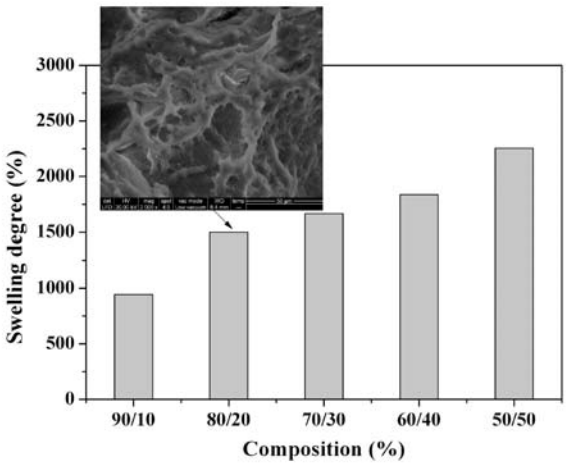

Fig. 4. Maximum swelling degree of PVA/P–CS semi-IPNs, in PBS (pH 7.4) at 37 °C (detail image: SEM micrograph of PVA/P–CS, 80/20 composition)

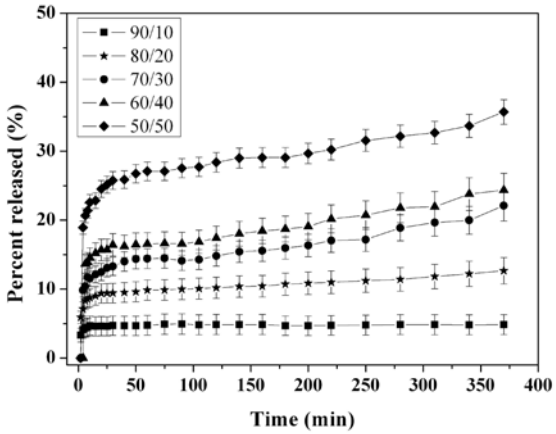

Fig. 5. Release profiles of metoprolol tartrate from PVA/P–CS semi-IPNs with different mixing ratio in PBS solution (pH 7.4) at 37 °C.

metoprolol tartrate from PVA/P–CS semi-IPNs was carried out in intestinal *p*H conditions at 37 °C. Figure 5 shows the cumulative metoprolol tartrate release from PVA/P–CS hydrogels in different mixing ratios. The release data are summarized in the table 1. The results obtained showed that the percent released was higher in case of hydrogels with an increased CS content. Thus, the formulations having less amount of CS (90/10 and 80/20) reached the maximum metoprolol tartrate release after 30 min, whereas in the case of the formulations having a higher amount of CS (70/30, 60/40 and 50/50) a controlled metoprolol tartrate release was observed, a rapid release within the first 40 minutes, followed by a decrease in the release rate. The interaction between drug and PVA/P–CS semi-IPNs mostly affected the releasing behaviour. The accumulation of metoprolol tartrate in the network could block the "micro porous" structure of the matrix decreasing the later flux. As the pore size of the compositions increased a higher cumulative metoprolol tartrate release was observed. The highest cumulative metoprolol tartrate release obtained at the end of releasing period (6 h) was 36% for 50/50 PVA/P–CS composition.

The values of the diffusion coefficient ranged from 0.11 to 0.24 indicating a Fickian mechanism [33] of the diffusion of metoprolol tartrate drug from the PVA/P–CS semi-IPNs. The release rate constant (k_r) increased with increasing of CS content in hydrogels composition.

Conclusions

Chondroitin sulfate based semi-interpenetrated polymer networks have been prepared using phosphorylated poly(vinyl alcohol). The swelling rate and equilibrium swelling were measured and correlated with the structure of the polymer matrices. The increase of the CS amount led to a higher macroporous structures resulting in an increase of swelling degree. The drug release profile can be controlled by modifying the ratio of semi-IPNs components.

Acknowledgements: The financial support offered to one of the authors (Dr. Tachita Vlad-Bubulac) by the European Social Fund – „Cristofor I. Simionescu" Postdoctoral Fellowship Programme (ID POSDRU/89/1.5/S/55216), Sectoral Operational Programme Human Resources Development 2007 – 2013, is gratefully acknowledged.

Table 1
KINETIC PARAMETERS OF METOPROLOL TARTRATE RELEASE FROM PVA/P–CS SEMI-IPNS

PVA/P–CS semi-IPNs	Korsmeyer-Peppas equation		
	n_r	$k_r 10^{-3}$ (min^{-n})	R
90/10	0.2	4.6	0.99
80/20	0.24	8.59	0.99
70/30	0.12	15.23	0.99
60/40	0.11	19.38	0.99
50/50	0.11	29.84	0.99

References

1.RAPOPORT, N., Progr. Polym. Sci., **32**, 8-9, 2007, p. 962.
2.STREHIN, I., NAHAS, Z., ARORA, K., NGUYEN, T., ELISSEEFF, J., Biomaterials, **31**, 10, 2010, p. 2788.
3.FISCHBACH, C., MOONEY, D. J., Adv. Polym. Sci., **203**, 2006, p. 191.
4.HOFFMAN, A. S., Adv. Drug Deliv. Rev., **64**, 2012, p. 18.
5.GRIFFITH, L. G., Acta Materialia, **48**, 1, 2000, p. 263.
6.AMIN, S., RAJABNEZHAD, S., KOHLI, K., Sci. Res. Essay, **4**, 11, 2009, p 1175.
7.HASSAN, C. M., PEPPAS, N. A., Adv. Polym. Sci., **153**, 2000, p. 37.
8.WANG, Y.-C., YUAN, Y.-Y., DU, J.-Z., YANG, X.-Z., WANG, J., Macromol. Biosci., **9**, 12, 2009, p. 1154.
9.HE, J., NI, P., WANG, S., SHAO, H., ZHANG, M., ZHU, X., J. Polym. Sci. Part A Polym. Chem., **48**, 9, 2010, p. 1919.
10.WACHIRALARPPHAITHOON, C., IWASAKI, Y., AKIYOSHI, K., Biomaterials, **28**, 6, 2007, p. 984.
11.LIU, J., HUANG, W., PANG, Y., ZHU, X., ZHOU, Y., YAN, D., Biomacromolecules, **11**, 6, 2010, p. 1564.
12.YANG, X.-Z., WANG, Y.-C., TANG, L.-Y., XIA, H., WANG, J., J. Polym. Sci. Part A Polym. Chem., **46**, 19, 2008, p. 6425.
13.ZHAO, Z., WANG, J., MAO, H.Q., LEONG, K. W., Adv. Drug Deliv. Rev., **55**, 4, 2003, p. 483.
14.IWASAKI, Y., AKIYOSHI, K., Biomacromolecules, **7**, 5, 2006, p. 1433.
15.WANG, D. A., WILLIAMS, C. G., YANG, F., CHER, N., LEE, H., ELISSEEFF, J. H., Tissue Eng., **11**, 1-2, 2005, p. 201.
16.KUIJPERS, A. J., ENGBERS, G. H. M., MEYVIS, T. K. L., de SMEDT, S. S. C., DEMEESTER, J., KRIJGSVELD, J., ZAAT, S. A. J., DANKERT, J., FEIJEN, J., Macromolecules, **33**, 10, 2000, p. 3705.
17.WANG, S.-C., CHEN, B.-H., WANG, L.-F., CHEN, J.-S., Int. J. Pharm., **329**, 1-2, 2007, p. 103.
18.DAWLEE, S., SUGANDHI, A., BALAKRISHNAN, B., LABARRE, D., JAYAKRISHNAN A., Biomacromolecules, **6**, 4, 2005, p. 2040.
19.VUJE, Z., RADULOVIC, D., AGBABA, D., J. Pharm. Biomed. Anal., **15**, 5, 1997, p. 581.
20.KRISHNAIAH Y. S. R., KARTHIKEYAN, R. S., SATHYANARAYANA, V., Int. J. Pharm., **241**, 2, 2002, p. 353.
21.VLAD-BUBULAC, T., SERBEZEANU, D., OPREA, A.-M., CARJA, I.-D., HAMCIUC, C., CAZACU, M., Cent. Eur. J. Chem., **11**, 3, 2013, p. 446.
22.GEFTER, A., In: Fosforganiceskie monomeri i polimeri Ed. Akad Nauk SSSR **1960**. p. 102.
23.BERENS, A. R., HOPFENBERG, H. B., Polymer, **19**, 5, 1978, p. 489.
24.SERBEZEANU, D., VLAD-BUBULAC, T., HAMCIUC, C., AFLORI, M., J. Polym. Sci. Part A Polym. Chem., **48**, 23, 2010, p. 5391.
25. RAFFAINI, G., GANAZZOLI, F., Macromol. Biosci., **7**, 5, 2007, p. 552.
26.PEPPAS, N. A., KORSMEYER, R. W., In: Hydrogels in Medicine and Pharmacy, Peppas N. A. Ed, CRC Press, Boca Raton, Florida; 1986, p.109.
27. KORSMEYER, R. W., PEPPAS, N. A., J. Control. Release, **1**, 2, 1984, p. 89.
28. SERRA, L., DOMENECH, J., PEPPAS, N. A., Biomaterials, **27**, 31, 2006, p. 5440.
29.PETREUS, O., VLAD-BUBULAC, T., CAZACU, G., Mater. Plast. (Bucharest), **42**, 2, 2005, p. 115.
30.BRIGHT, A., RENUGA DEVI, T. S., GUNASEKARAN, S., Int. J. ChemTech Res., **2**, 1, 2010, p. 379.
31.DUMITRIU, R. P., MITCHELL, G. R., VASILE, C., Polym. Int. **60**, 2011, p. 222.
32.COMPER, W.D., ZAMPARO, O., Biochem. J., **269**, 1990, p. 561.
33.HEKMAT, A., BARATI, A., FRAHANI, E. V., AFRAZ, A., World Acad. Sci. Eng. Technol., **56**, 2009, p. 96.

Manuscript received: 13.02.2103