Poly(ethylene Glycol) Diacrylate-Nanogels Synthesized by Mini-emulsion Polymerization

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New nanogels (NGs) with tailored properties were obtained using a mini-emulsion technique, from poly(ethylene glycol) diacrylate (PEGDA) self-crosslinking macromers of various molecular weight. By modifying synthesis parameters (hydrophilic-lipophilic balance, emulsifier and the ratio of organic-aqueous medium), optimum recipes of NGs were selected. Therefore, the molecular weight distribution and the functionalization degree of the $PEGDA_{2000}$ macromer were assessed by Gel Permeation Chromatography (GPC) and Nuclear Magnetic Resonance (NMR), respectively. Furthermore, the PEGDA-NGs were investigated by Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM) for size distributions and morphology. DLS and TEM results confirm that these new PEGDA-NGs hold potential for biomedical applications.

Keywords: poly(ethylene glycol) diacrylates macromer, self-crosslinking, mini-emulsion, nanogels

Hydrogels are three-dimensional hydrophilic networks able to swell and to retain large amounts of water within their structure, without dissolution [1-3]. During the last few decades, most research was focused on the obtaining macroscopic hydrogels, but, nowadays, micro- and nano-sized hydrogels are paid a great deal of attention, being also known as micro- and NGs [4,5]. Yet, their submicron size endows them with unique properties like the ability to permeate the cellular membranes, which renders them suitable for subcutaneously or intravenously administration reaching inaccessible areas. Most common applications of such NGs, with controlled release properties of various active principles refer to the tissue engineering field, biomedical implantology and bionanotechnology [4]. Other advantages of the NGs over the bulky macroscopic hydrogels [4,6] refer to a larger surface area highly required for in vivo applications and to the ability to embed compounds with various molecular weights.

The most frequent methods used for micro- and NGs synthesis are usually heterogeneous polymerization processes like (i) *polymerization in inverse mini- or microemulsion (oil -water),* which involves a stable mixture composed of water-soluble polymer-surfactant assemblies in a continuous organic medium [6], and (ii) *precipitation polymerization* that begins as a continuous phase, in which the monomer and the initiator are completely soluble, followed by polymer growth and precipitation as the molecular weights increase [7]. The latter method presents several drawbacks i.e. low yields of preparation, irregular particle shapes and high polydispersity indexes (PDI) [8].

Literature studies show the diversity and versatility of such systems, optimized for drug delivery. Park and Yoo [9] have synthesized new NGs structures based on bifunctional polyethylene glycol amine and carboxylic acid with the capacity of entrapping doxorubicin (DOX). Shimoda and coworkers [10] obtained polyethylene glycol (PEG)/ polysaccharides hybrid NGs, to be used as nano-carriers and controlled release systems for proteins. Literature also shows that, among various synthetic polymers, poly (ethylene glycol) (PEG) is the most used precursor for NGs. Many applications in the biomedical field, particularly in drug delivery and tissue engineering use NGs based on PEGDA, given their remarkable properties and characteristics, like biocompatibility and hydrophilicity, which can be exploited for site specific controlled drug delivery [11].

Consequently, this study is aimed at obtaining new NGs exhibiting tailored properties in terms of size and hydrophilicity. In this respect, a PEGDA macromer was designed by functionalizing PEG_{2000} oligomers with acrylate end-groups *via* the acryloyl chloride method. This step was followed by the synthesis of NGs using inverse mini-emulsion techniques, which consisted of dispersing the aqueous solution of reactants in an organic phase in the presence of sorbitan monooleate and polyethylene glycol sorbitan monostearate, as emulsifiers. In our approach, the synthesized $PEGDA_{2000}$ macromer and a commercial $PEGDA_{700}$ macromer were self-crosslinked, either separately or as blends, in the presence of tetramethyl-ethylenediamine and ammonium persulfate. To the best of our knowledge, such PEGDA NG structures obtained using the synthesis methodology described previously and the two employed self-crosslinking macromers, used alone or as blends, has not been reported in the literature until now [12-17]. The important differentiator of the proposed synthesis path refers to the only use of self-crosslinking PEGDA macromers without additional short-chain crosslinkers, like methylene bisacrylamide (MBA) [18].

Onward the focus within this paper will be on optimizing the recipes for NGs synthesis. To this end, important parameters of mini-emulsion, such as: the hydrophilic-lipophilic balance of the surfactant (HLB), the surfactant/ aqueous and the solvent/aqueous medium ratio, the monomer concentration, the molecular weight of the PEGDA oligomer and the blending ratio of the two different

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macromers ($PEGDA_{700}$ and $PEGDA_{2000}$) were studied for quantifying their effects upon the particle size, size distribution and morphology of these advanced NGs.

Experimental part

Chemicals

Polyethylene glycol (MW=2000 Da) (PEG_{2000}, Fluka, 99%), benzene (Sigma-Aldrich, 99%), acryloyl chloride (AcCl, Alfa Aesar, 96%), triethylamine (TEA, Merck, 99%), methanol (Scharlau, 99.9%), methylene chloride (Sigma-Aldrich, 99.9%) and diethyl ether (Sigma Aldrich, 99.8%) were used, for macromer syntheses, without further purification. $PEGDA_{700}$ (MW=700 Da) (Sigma-Aldrich), cyclohexane (Sigma-Aldrich, 99.5%), tetramethylethylenediamine (TMEDA, Merck, 99%), ammonium persulfate (APS, Peking Chemical Works, 98%), sorbitan monooleate (Span 80, Sigma-Aldrich) and polyethylene glycol sorbitan monostearate (Tween 60, Fluka) were employed for NG synthesis and were used as received.

Functionalization of PEG

PEG_{2000} was functionalized with acrylate end-groups, obtaining PEGDA, by well-known synthesis routes [19]. The method used 14 g of PEG_{2000} (or PEG_{700}) which where dissolved in 160 mL benzene from which 100 mL were removed from reaction after an azeotropic distillation at 80 °C. After cooling, 12 mL TEA and 7 mL AcCl were added dropwise to the PEG_{2000} solution. The resulting solution was bubbled for 30 min with nitrogen, in order to remove the traces of humidity and stirred for 70 h at 30 °C. At the end of the reaction time, 5.2 mL methanol was added, and the mixture was stirred for another 2 h. The insoluble triethylamine hydrochloride salt was removed by filtration. The $PEGDA_{2000}$ macromer was purified according to the procedure described in the literature [20].

Hydrogel nanoparticles preparation and purification

The synthesis consisted of self-crosslinking of the macromers *via* an inverse-phase emulsion technique. The NGs were prepared using the recipes described in table 1. In brief, $PEGDA_{2000}$ and $PEGDA_{700}$ macromers were self-crosslinked separately or as blends, according to the values described in table 1. In the PEGDA blends the commercial product of $PEGDA_{700}$ was used. First, the macromer was dissolved in distilled water, together with TMEDA (accelerator of the cross-linking process). The organic medium was prepared separately by mixing the nonpolar cyclohexane solvent with the emulsifier system [Span 80/Tween 60 in various ratios- to assess the influence of the hydrophilic-lipophilic balance (HLB)]. Both the aqueous and the organic mixtures were purged with N_2 in order to remove O_2 that might inhibit the free radical reaction [21], and the aqueous phase was injected drop-wise into the reaction vessel. Finally, 20% APS solution was added to the mixture and left for 42 h, under stirring at 30 °C, until the reaction was complete. The initial stirring speed was initially of 1300 rot/min, and then was gradually reduced during the synthesis, in order to minimize the coagulum quantity. The HLB was varied by means of modifying the ratio between the two emulsifiers in the blend, and calculated with Eq. (1):

$$HLB_{blend} = \sum_{i=1}^{n} x_i \times HLB_i \quad (1)$$

where x_i is the mass fraction of surfactant i and HLB_i is the corresponding HLB value.

At the end of reaction, the particles were washed and separated by centrifugation, as follows: washed twice with 45 mL of cyclohexane (the supernatant liquid collected for sampling), twice with 45 mL acetone and twice with 45 mL distilled water, each cycle for 30 minutes at 9000 rpm. The final solid xerogel was obtained by lyophilization of washed hydrogel particles.

Sample	Solvent/Aqueous, % wt.	Emulsifier/Aqueous, % wt.	HLB[a]	$PEGDA_{2000}$/$PEGDA_{700}$, % wt.
E1	5.11	3	6.95	100/0
E2	6.60	1.5	6.95	100/0
E3	10	3	6.95	100/0
E4	10	1.5	6.95	100/0
E5	5.11	3	5.89	100/0
E6	**5.11**	**3**	**5.625**	**100/0**
E7	**5.11**	**3**	**5.625**	**0/100**
E8	**5.11**	**3**	**5.625**	**25/75**
E9	5.11	3	5.625	50/50
E10	5.11	3	5.625	75/25
E11	5.11	1.5	6.95	100/0
E12	6.60	3	6.95	100/0
E13	5.11	3	5.36	100/0
E14	6.60	3	5.625	100/0
E15	10.00	3	5.625	100/0
E16	5.11	3	5.89	0/100
E17	5.11	3	6.95	0/100

[a] hydrophilic-lipophilic balance (HLB)

Table 1
INVESTIGATED PARAMETERS FOR THE PEGDA-NGs SYNTHESIS

Characterization methods

Proton Nuclear Magnetic Resonance (H^1-NMR). The PEGDA macromer was analyzed by H^1-NMR to determine the functionalization degree, with the use of a Bruker Advance III Ultrashield Plus 500 MHz spectrometer, working at 11.74 T, with deuterated chloroform ($CDCl_3$) as solvent.

Gel Permeation Chromatography (GPC).

Chromato-grams of PEG_{2000} precursor and of the obtained macromer were registered using the HPLC 1200 Series with refractive index detector (RID) from Agilent Technologies equipped with a PLGel Mixed-C (300×7.5 mm) Column and isocratic pump. Dimethylformamide was used as mobile phase at a 1 mL/min flow rate. The column was calibrated using Polyethylene oxide/glycol EasiVial standards in the 106-1522000 g/mol range of molecular weight (M_n).

Dynamic Light Scattering (DLS).

Size distribution and polydispersity index (PDI) for particles were determined by DLS using Zetasizer Nano ZS, ZEN3600, with a 4 mW and 633 nm wavelength He-Ne laser, measuring range being of 0.6 nm-6μm.

Bright Field Scanning Transmission Electronic Microscopy (BFSTEM).

BFSTEM images were collected using Tecnai™ G2 F20 TWIN Cryo-TEM Instrument and employed to confirm the size of NGs and asses their morphology. In this respect, the emulsions in cyclohexane are left to dry on a carbon film grid. The excess of surfactant is then removed by dipping the grids for 5 s in acetone. BFSTEM images of the NGs after purification process were recorded at an acceleration voltage of 200 kV, after confirmation that the emulsion/NGs morphology is not affected by the large exposure at the given acceleration voltage.

Results and discussions

$PEGDA_{2000}$ Macromer Characterization

GPC measurements

In order to investigate the functionalization process, the $PEGDA_{2000}$ macromer was characterized by GPC in terms of number average molecular weight (Mn) and polydispersity index (PI= Mw/Mn). The molecular characteristics of thereof are given in table 2. The PI of the macromer presented low values (PI<1.5) which indicated a well controlled functionalization process of $PEGDA_{2000}$. One may notice from table 2 that the PEG_{2000} alone showed lower Mn values in comparison to the functionalized PEG. This fact also indicated that the PEG_{2000} backbone did not suffer destruction or other major structural changes during the synthesis. Hence, the functionalization reaction is straightforward. Furthermore, to sustain the results, in figure 1 it can be noticed that the molecular weight distributions of PEG_{2000} and $PEGDA_{2000}$ were similar and monomodal.

Table 2

GPC RESULTS OF PEG PRECURSOR AND OF THE SYNTHESIZED MACROMER

Sample	PI[a]	$M_n \cdot 10^5$ (gmol^{-1})
PEG_{2000}	1.14	1.294
PEGDA	1.11	1.455

[a] Polydispersity index of the macromer, PI=M_w/M_n given by GPC

H^1- NMR measurements

The functionality for the $PEGDA_{2000}$ macromer was determined according to Eq. (2) by comparing the peaks area corresponding to methylene (CH_2) group protons (δ = 6.44) with the protons of the oxyethylene groups (CH_2CH_2O - δ = 3.65) from the ^{1}H-NMR spectra, as shown in figure 2.

$$\bar{f}(acrylic\ groups) = \frac{A_{methylene}}{A_{oxyethylene}/4} \times \overline{DP_{nPEG}} \qquad (2)$$

The synthesis route of the PEGDA macromer registered a 1.87 functionality value and, thereby, a 93% functionalization yield. The functionality is vital for generating multiple cross-linking nods that lead to the formation of robust hydrogel matrix.

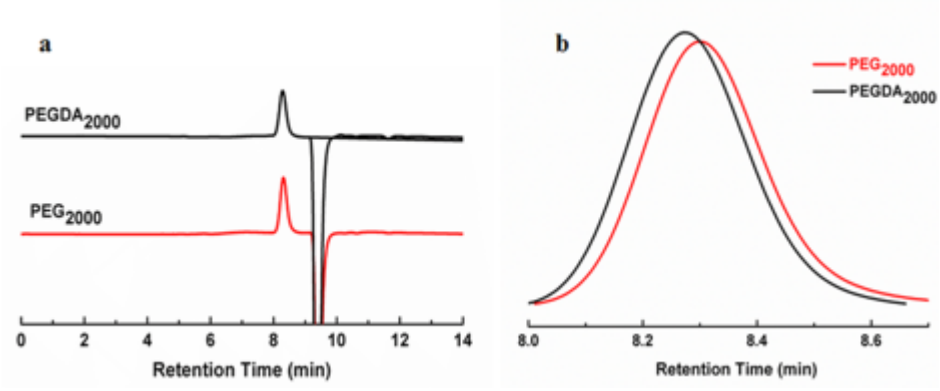

Fig. 1. Elugrams (a) and GPC traces (b) of PEG_{2000} precursor and functionalized macromer

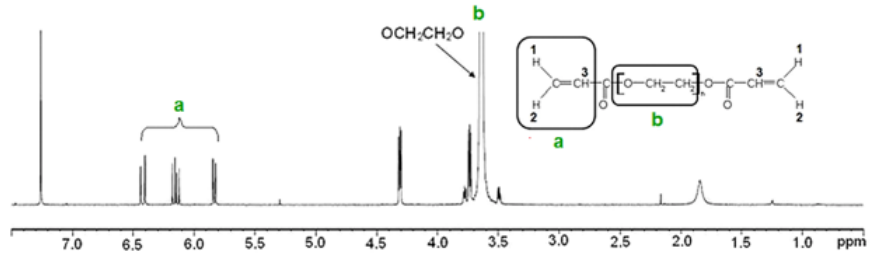

Fig. 2. ^{1}H-NMR spectra for the new macromer

NGs characterization

DLS measurements

A summary of the DLS results for the analyzed samples is presented in Table 3. The best results in terms of nanoparticle size were obtained for the following values of studied parameters: HLB balance 5.625, molar ratio between monomers $PEGDA_{700}/PEGDA_{2000}$ = 75/25 (corresponding to $MW_{mixture}$ of 1025 Da), monomer concentration = 22%, initiator concentration = 5%, the ratio between surfactant and aqueous phase = 3, and the ratio between solvent and aqueous phase = 5.1.

Table 3

DLS PARAMETERS OF RESULTED NGs

Sample	Average size, nm	PDI[a]
E1	329.4	0.569
E2	593.50	0.870
E3	675.00	0.921
E4	499.30	0.713
E5	244.90	0.304
E6	**161.20**	**0.197**
E7	**157.10**	**0.011**
E8	**150.90**	**0.004**
E9	158	0.182
E10	160	0.194
E11	504.40	0.524
E12	588.00	0.568
E13	244.00	0.278
E14	286.00	0.36
E15	328.00	0.4
E16	210.00	0.182
E17	183.00	0.089

[a]Polydispersity index of particles, *(width/mean)^2* given by DLS

The influence of the medium composition upon the particles size, size distribution and PDI for the resulted NGs

Further on, the influence of the emulsifier/aqueous medium and that of the ratio solvent/aqueous medium was evaluated and shown in figure 3. For the same reaction conditions, increasing the emulsifier amount, with respect to the macromer/aqueous phase, influenced the PDI and, implicitly, the stability of the emulsion, rather than the average size of particles.

At the same time, the ratio between the solvent and the aqueous medium influenced both the size and the stability of the emulsions, to some extent. However, by maintaining the wt. ratio of emulsifier system/disperse phase constant, when increasing the amount of solvent, a slight increase in the NGs size was noticed. This behavior is due to the low concentration of the emulsifier in the reaction mixture, accounting for the interfacial tension of the droplets; low interfacial tensions expressing as an overall higher tendency of NGs to coalescence.

The influence of the $PEGDA_{2000}$ macromer and initiator extent upon the particles size, size distribution and PDI for the resulted NGs

In order to investigate the influence of the macromer concentration upon the NGs particle size, a part of the syntheses was carried out using a macromer concentration of 22 wt.%, and a few with either 12 wt.% or 29 wt.% (according to Table 1). Similarly, the influence of the initiator concentration upon the particle size of the NGs was investigated. In this respect, the concentration of the initiator was varied from 2.36 wt.% (in most cases) and 4.72 wt.% (for sample E17). According to the literature, the size of the NGs increased with the monomer concentration [22]. However, in our study lower monomer concentrations slightly increased the particle size of the NGs, according to figure 4. This can be explained by the formation of some intramolecular dangling chains and loops [23], which might cause some steric repulsive forces with a potential effect of particle aggregation [22]. The lowest particle size corresponded to a monomer concentration of 22 wt.%, and, consequently, the rest of the experiments were conducted using this monomer concentration. A similar evolution was observed for the PDI, as well.

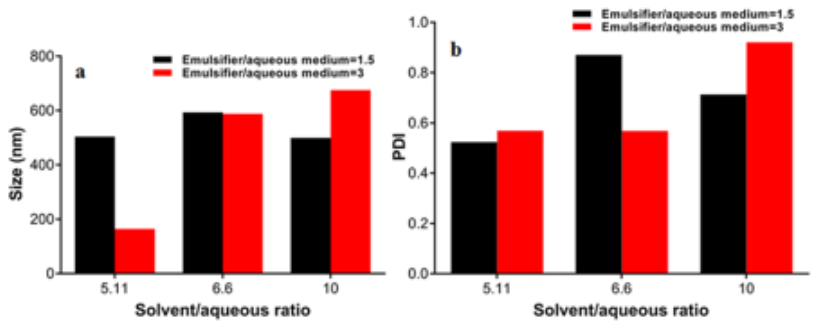

Fig. 3. Size distribution (a) and polydispersity index (b) of particles obtained by variation of solvent/aqueous medium ratios and emulsifier/aqueous ratios

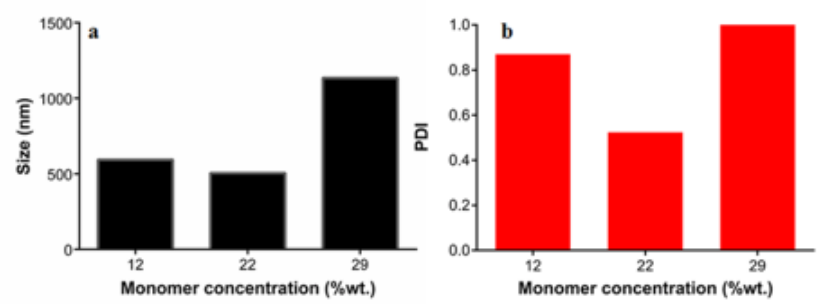

Fig. 4. Size distribution (a) and polydispersity index (b) of particles obtained by variation of ($PEGDA_{2000}$) macromer concentrations

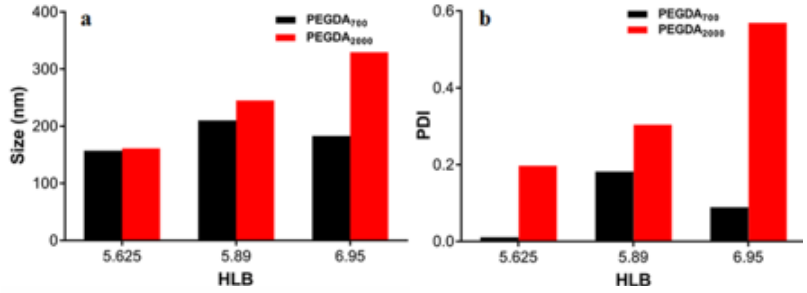

Fig. 5. Size distribution (a) and polydispersity index (b) of particles obtained from starting material $PEGDA_{700}$ respectively $PEGDA_{2000}$ by variation of HLB

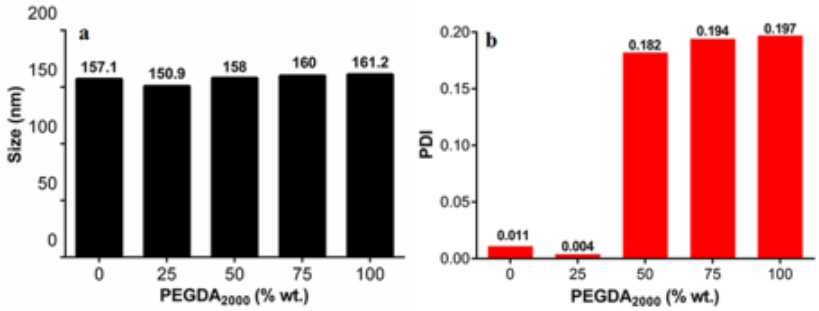

Fig. 6. Size distribution (a) and polydispersity index (b) of particles obtained by the variation of blending ratio between $PEGDA_{700}$ and $PEGDA_{2000}$

The influence of macromers type ($PEGDA_{2000}$, $PEGDA_{700}$ or blend) upon the particles size, size distribution and PDI for the resulted NGs

When varying the HLB of the system, by means of changing the Span 80/Tween 60 ratio, a linear trend was observed for the size of the NGs with $PEGDA_{2000}$, as shown in figure 5a. This behavior may be attributed to the different solubility in water of the Span 80/Tween 60 mixtures that generates instability in the sample at greater HLB values and causes the nanoparticles to agglomerate, as indicated by the rather broad size distributions. Our results fit well with other literature studies regarding the optimal HLB for W/O miniemulsions, which varies from 4 to 6, as the optimum diameter of the NGs prepared in draft are about 150 nm at an HLB of 5.625. Therefore, for emulsions with hydrosoluble monomers in nonpolar organic phase, like our case, emulsifiers with a low HLB value are highly recommended [22].

Emulsions composed of $PEGDA_{700}$ yielded slightly smaller particles for the optimum HLB interval (between 4 and 6) than the corresponding ones that used $PEGDA_{2000}$. When looking at the PDI (fig. 5b), it can be noticed that the distribution of the $PEGDA_{2000}$-based NGs (PDI=0.569) is considerably broader than the one of $PEGDA_{700}$ (PDI=0.089) at an HLB value of 6.95. This was expected, as the NGs were obtained by self-crosslinking, and shorter polymeric chains lead to denser networks. Another possible explanation for the increase in the particle size of $PEGDA_{2000}$, as compared to the $PEGDA_{700}$, may refer to a greater viscosity of the continuous phase during the hydrogel synthesis. According to other literature studies, the high particle size of NGs is also due to the superior length of the solvated PEG chains tethered to the nanogel particle surface [24]. This hypothesis is valid for all the cases described above and, together with the fact that the difference in size (percentile wise) is much lower than the difference in PDI (percentile wise), leads to the conclusion that the main influence of the molecular weight (and dimension, implicitly) of the macromer is more related to the size-distribution, rather than the size itself. At the same time, it can be concluded that size and PDI is adjustable by choosing various HLB ratios of the emulsifier blend.

It is also possible to tailor the composition and properties of the NGs by modifying the ratios between the two oligomers with different chain length ($PEGDA_{700}$ and $PEGDA_{2000}$) [23,25]. Due to the particularity of PEGDA, which displays the ability to act both as a monomer and as cross-linker [26], the mixture between a macromer with short length ($PEGDA_{700}$) and one with longer chains ($PEGDA_{2000}$) was used to generate NGs with special architecture. The rationale behind the proposed mixture refers to increasing the hydrophilicity of the NGs by using higher molecular weight PEG and decreasing the particle size by using low molecular weight PEG [24] Hence, preparing NGs using two polymeric building blocks with different hydrophilicity, mobility, and biocompatibility (greater for $PEGDA_{2000}$) [27-30] constitutes a mechanism for tuning the hydrophilicity and size of particles. For instance, tailoring more versatile NPs is highly important for drug delivery applications where the drugs differ by hydrophilicity and the release mechanism of such drugs is mainly influenced by the interactions with the NP carriers.

As shown in figure 6, combining the two macromers yielded promising results. The possible mechanism for generating these types of NGs can rely on $PEGDA_{2000}$ in lower proportion to act as a crosslinker, while the other one provides the backbone of the hydrogel. DLS analysis revealed that the addition of a small amount of $PEGDA_{2000}$ (25 wt.% relative to the PEGDA blend), in the reaction mixture, yields NG particles with small sizes of about 151 nm and nearly unimodal distribution, PDI=0.004.

Morphology of resulted NGs

TEM images (fig. 7) of the $PEGDA_{2000/700}$-based NGs from emulsion and after purification provided important information regarding the size, size distribution and the

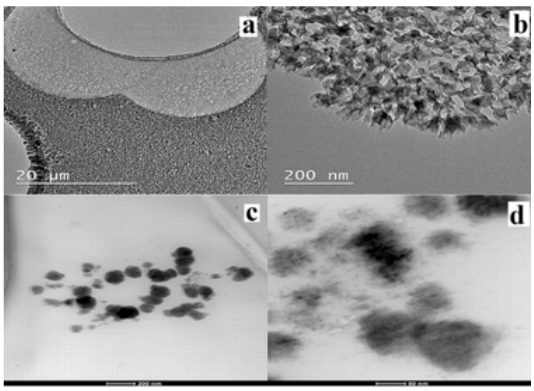

Fig. 7. TEM images of the $PEGDA_{2000/700}$-based NGs: in emulsion (a- 20µm; b- 200 nm) and after purification (c- 200 nm; d- 50 nm)

aggregation degree of NGs. This result served as confirmation for some of the dimensional details obtained through DLS technique, particularly for the NGs with 25 wt.% $PEGDA_{2000}$ relative to the $PEGDA_{2000/700}$ blend. The micrographs in figure 7a and b revealed agglomerates of nanoparticles in emulsion with high particle sizes due to the presence of emulsifiers at the surface. After purification (fig. 7c and d), the NGs presented a spherical morphology, rather porous, with dimensions roughly ranging from 60 to 160 nm and an average size of about 115 nm. It is also important to point out that NGs after purification were less agglomerated as a result to the gentle washing procedure and immediate lyophilization.

Conclusions

Innovative NGs were successfully obtained by a mini-emulsion technique starting from a self-crosslinking macromer of $PEGDA_{2000}$ obtained by the acryloyl chloride method. GPC and H^r-RMN measurements indicated that $PEGDA_{2000}$ was proper in terms of functionality and high yield, creating the premises for the formation of well-defined hydrogels matrixes. Optimization of NGs preparation was accomplished by studying various parameters specific for the mini-emulsion polymerization with the aid of DLS and TEM. The two latter techniques revealed that the NPs sizes ranged from 150 to 675 nm, with spherical morphologies and rather porous. In order to increase the hydrophilicity and lower the size and size distribution of NGs, a synergic mixture of $PEGDA_{2000}$ and $PEGDA_{700}$ was used to generate spherical 151 nm sized NGs with a 0.004 PDI, which were also rather independent after purification and lyophilization (making the NGs redispersible). Hence, preparing NGs using two polymeric building blocks with different hydrophilicity and mobility constituted a mechanism for tuning the essential properties of NGs.

Acknowledgements. The contribution of Lecturer dr. Sorin Avramescu (University of Bucharest, Faculty of Chemistry) who provided the H NMR results and of Phd student Bogdan Trica (INCDCP-ICECHIM Bucharest) who performed the TEM images is gratefully acknowledged. This work was supported by the PN III- Human Resources Program - YOUNG RESEARCH TEAMS –PN-III-P1-1.1- TE-2016-1876, grant no. 91/ 2018 and by the Romanian Research Projects PCCDI no. 39/2018– INTELMAT.

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Manuscript received: 13.06.2019