Intermediary Scientific Report

For the project “Porous ionic matrices with tailored architectures and responsiveness to host bioactive compounds”

Cod: PN-II-ID-PCE-2011-3-0300

on the project implementation between January 2012 - November 20th, 2013

Three of the project objectives have been solved between January 2012 – November 20th, 2013, the obtained results being presented below.

Objective #1: Construction of ionic thin films by layer-by-layer technique and the evaluation of their sorption/immobilization potential of some bioactive compounds using as building blocks synthetic or natural ionic or ionizable polymers.

Solving this objective, cationic monocomponent and dicomponent (polycation/polyanion) polyelectrolyte multilayers, capable to immobilize and release bioactive compounds (enzymes, model dyes), have been obtained by the layer-by-layer deposition of polyelectrolytes from aqueous solutions.

1.1. Polycationic multilayers thin films based on synthetic polycations

For the construction of this new type of films the following components have been used: poly(ethyleneimine), a weak polycation ionized on a large scale of pH, two bifunctional cross-linkers, 3,3',4,4'-benzophenonetetracarboxylic dianhydride (BTCDA) and glutaraldehyde (GA), different silica substrates and two enzymes, lysozyme (LYS) and pepsin (PEP). Using layer-by-layer assembly through hydrogen bondings and electrostatic interactions mediated by BTCDA, it was obtained single polycation cross-linked multilayers based on linear poly(ethyleneimine) [PEI(L)] and branched poly(ethyleneimine) [PEI(B)]. Polycations were adsorbed from salt-free aqueous solutions onto: (i) silica microparticles (Daisogel type) with particle diameter of 40 - 60 µm and pore dimensions of 100 nm; (ii) silica microparticles (Davisil type), with 9 – 11 µm diameter and pores of 5 -7 nm; (iii) silicon wafers with surface of 1 cm². For the stabilization of PEI(L) and PEI(B) macromolecules onto solid surface of silica, a cross-linking with BTCDA was realized, resulting a solid surface covered with free carboxylic groups. The cross-linked layers, which are negatively charged over a wide range of pH, can adsorb a new polycation layer. Construction strategy of single component cationic films and enzymes sorption onto their surface is illustrated in Scheme 1.

Scheme 1
As can be seen in Figure 1, the zeta-potential measurements have shown a regular increase of the single component multilayer thin films, both for PEI(L) (left) and PEI(B) (right). Before enzymes immobilization, the third polyelectrolyte layer was suplimentary cross-linked and activated by GA.

**Figure 1.** Zeta-potential values determined from streaming potential measurements as a function of pH of the aqueous KCl solution ($C_{KCl} = 10^{-3}$ mol/L) for the Daisogel microparticles modified with multilayers of PEI(L) (left) and PEI(B) (right).

After the adsorption of the third polymer layer, the cross-linked polycationic multilayers deposited onto silica microparticles were additional cross-linked with GA in excess to generate free aldehyde groups, which were used in LYS and PEP immobilization. In Figure 2 is presented the zeta-potential variation for composite Daisogel microparticles modified with polycationic multilayers of PEI(L) (left) and PEI(B) (right) after the GA cross-linking and immobilization of LYS and PEP.

**Figure 2.** Zeta-potential values, determined by streaming potential measurements for composite Daisogel microparticles modified with single component multilayers of (a) PEI(L) and (b) PEI(B) after the GA cross-linking and immobilization of LYS and PEP, as a function of pH of a aqueous KCl solution ($C_{KCl} = 10^{-3}$ mol/L).
From Figure 2 we can see that after the GA cross-linking, the \( iep \) values of Daisogel composite microparticles modified with PEI(L) and PEI(B) multilayers decreased to approximately 7.5. After LYS immobilization, the \( iep \) didn’t change significantly, showing that LYS has been immobilized in a very small amount onto composite microparticles. On the other hand, after the PEP immobilization, the \( iep \) value of Daisogel composite microparticles modified with PEI(L) and PEI(B) multilayers decreased to approximately 6.7 and 6.4, respectively, suggesting that PEP was immobilized in a higher amount onto composite surface.

Alternately adsorption of the polycation, cross-linking with BTCDA and GA, as well as the enzyme immobilization onto single component cross-linked polyelectrolyte multilayers introduced considerable amounts of carbon and nitrogen onto solid surface of silica microparticles. As a consequence, elemental quantitative analysis of the thin organic film has been carried out using X-ray photoelectron spectroscopy (XPS), after each step (adsorption/cross-linking/immobilization). From \([C]:[Si]\) and \([N]:[Si]\) atomic ratios values it was concluded that PEI(L) and PEI(B) films increased with the increasing of the number of cross-linked layers. After the PEP and LYS immobilization onto composites surface, the atomic ratios \([C]:[Si]\) and \([N]:[Si]\) showed that PEP was immobilized in a higher amount than LYS, due to the different isoelectric points of those two enzymes. Thus, the immobilization process of PEP (\( iep \approx 1 \)) was favored by the electrostatic interactions with the cross-linked organic film, while the LYS immobilization (\( iep \approx 10 \)) was not favored by the electrostatics between it and polycation.

The amount of immobilized PEP onto cross-linked multilayers based on PEI(B) deposited onto Daisogel microparticles was also followed as a function of the number of polycation adsorbed layers. After the adsorption of each PEI(B) layer, a GA cross-linking was carried out to generate free aldehyde groups to interact with PEP (Scheme 2).

In Figure 3 are presented the \([C]:[Si]\) and \([N]:[Si]\) atomic ratios determined from XPS spectra of composite Daisogel microparticles after adsorption of each PEI(B) layer and GA cross-linking (closed symbols), as well as after the PEP immobilization (open symbols). As can be seen, the \([C]:[Si]\) and \([N]:[Si]\) atomic ratios increased with the number of PEI(B) adsorbed and cross-linked layers, showing the formation of cross-linked multilayers. PEP immobilization onto PEI(B) cross-linked multilayer was followed by the increasing of \([C]:[Si]\) and \([N]:[Si]\) atomic ratios after each adsorbed polymeric layer.

The surface characteristics (average high, \( h_a \), and average roughness, \( R_a \)) determined by atomic force microscopy (AFM), slightly increased after each modification of the film.
This results are in agreement with XPS analysis, showing that the film with the highest organic amount deposited onto Daisogel microparticles had the highest $h_a$ and $R_a$ values among all films deposited onto silicon wafers.

This new type of single component multilayer thin film based on PEI could be used in functionalization of solid surfaces for subsequent deposition of new layers, for immobilization of bioactive compounds or creation of some surfaces with a balance between different types of interactions: hydrogen bonds, electrostatic interactions and/or hydrophobic interactions. The enzyme immobilization onto this kind of thin films shows the potential application in immobilization of other biomolecules, synthesis of composite membranes, new systems for controlled drug delivery etc.

1.2. Natural polycation/synthetic polyanion porous multilayer films

New porous multilayer films based on chitosan (CS) as polycation and poly(acrylic acid) (PAA) as polyanion have been constructed by layer-by-layer deposition method. After their construction, the multilayer films were post-treated as follows: post-treatment $T_1$, which consisted of immersing the multilayer films in water with $pH = 2.4$ for 1 min, followed by immersion in pure water for 15 s and drying at $80^\circ C$ for 60 min; post-treatment $T_2$, which consisted of immersing the multilayer films in pure water for 30 min and drying at $120^\circ C$ for 60 min; post-treatment $T_3$, which consisted of immersing the multilayer films in water with $pH = 2.4$ for 5 min, followed by immersion in pure water for 1 min and drying at $120^\circ C$ for 60 min; post-treatment $T_4$, which consisted of post-cross-linking of CS chains with glutaraldehyde (GA). The influence of these post-treatments on the topography, thickness, roughness and hydrophilicity of the multilayer films was investigated by atomic force microscopy (AFM), ellipsometry and contact angle measurements.

Figure 4 (left) shows the AFM images of (CS/PAA)$_{5.5}$ multilayer film, before and after post-treatments $T_2$ and $T_3$. AFM images, B and C (Figure 4, left) show the morphological modifications of the multilayer film after the $T_2$ and $T_3$ post-treatments, due to the modification of medium $pH$ and ionic strength. The cross-section profiles drawn in the AFM images A, B and C are compared in Figure 4D (left). In the case of CS/PAA multilayer film immersed in water with $pH = 5.5$, without NaCl (post-treatment $T_2$), the distance between the spherical formations onto its surface was similar to that of the untreated film. The immersion of the film in an aqueous solution with $pH = 2.4$, without NaCl, led to the appearance of channels between the spherical formations onto the surface, having the width of approximately 100 nm, which suggests that the $pH$ variation had a more pronounced effect on the

![Figure 3](image-url) [C]:[Si] and [N]:[Si] atomic ratios determined from XPS spectra of Daisogel microparticles modified with PEI(B) after GA cross-linking (closed symbols) and PEP immobilization (open symbols).
morphological modifications of the CS/PAA multilayer film then the lack of NaCl in post-treatment solutions.

**Figure 4.** AFM images (1 x 1 µm²) of (CS/PAA)₅₅ multilayer film deposited onto silicon wafers from solutions with C_polymer = 10⁻² mol L⁻¹ and C_NaCl = 10⁻¹ mol L⁻¹, before (A) and after post-treatments T₂ (B) and T₃ (C). Image D shows the cross-section profiles drawn in images A, B and C.

The sorption/release capacity for bioactive compounds of the CS/PAA multilayer films was tested using two cationic dyes, Methylene Blue (MB) and orto-Toluidine Blue (TBO), which are known for their biological properties. TBO is a metachromatic dye possessing an increased affinity for acid components of the living tissues rich in DNA and RNA, being used in vivo for the detection of dysplasia and carcinogenic tissue in the oral cavity. MB is used in surgery to identify tissues which will be removed, to identify pre-carcinogenic lesions in gastrointestinal tract, or for strengthening the cements used to repair bone tissues. The results obtained by studying the multilayer films deposited onto silicon wafers showed that post-treatment T₃ produced the most significant morphological modifications of the multilayer’s surface. Therefore, to evaluate sorption/desorption capacity for the two model dyes, CS/PAA multilayer films have been constructed onto Daisogel microparticles using the same deposition conditions, after which they were subjected to post-treatment T₃. The investigation of the sorption kinetics showed that the equilibrium was established in approximately 60 min. Figure 4 (right) shows the MB sorption isotherms on Daisogel/(CS/PAA)₅₅, before and after the post-treatment. The experimental data have been fitted by nonlinear regression method using two model isotherms, Langmuir and Freundlich. As Figure 4 (right) shows, the amount of dye sorbed at equilibrium was higher for the untreated multilayer film than for the treated one. However, the net advantage of these multilayer films is their reusability in three consecutive sorption/desorption cycles, while the untreated multilayer delaminated after the first regeneration.

The results connected with this objective have been published in two ISI journals:


Objective #2: Design of novel porous ionic multifunctional (PIM) hydrogels based on poly(acrylamide) and natural ionic polymers by cryogelation (cryogels type IPN)

Hydrogels, as materials similar with living tissues, are able to respond to various external stimuli, and therefore have found numerous biomedical applications in drug delivery systems, tissue engineering, biosensors, actuators, etc. In applications like artificial implants and drug delivery systems, multicomponent hydrogels such as semi-interpenetrating polymer networks (semi-IPN) and IPN demonstrated a faster response rate at the external stimuli and a better diffusion of the bioactive compounds. A special interest lately focused on the macroporous hydrogels, due to their much faster response rate at the external stimuli compared to the conventional hydrogels. To prepare novel hydrogels based on poly(acrylamide) (PAAm) and natural ionic polymers, semi-IPN hydrogels have been first prepared by cryogelation, using either chitosan (CS) or a polyanion derived from potato starch as polymer entrapped in the PAAm matrix.

2.1. Synthesis and characterization of the IPN composite cryogels based on PAAm and CS

Synthesis of some novel semi-IPN ionic composite hydrogels based on PAAm and CS was performed by cryogelation technique. IPN ionic composite hydrogels have been obtained by cross-linking the CS chains with epichlorohydrin (ECH), in alkaline medium (Figura 5), a partial hydrolysis of amide groups taking place in these conditions, with the simultaneous generation of some COO⁻ groups.

![Figure 5. Formation of IPN composite cryogel having two oppositely charged networks by cross-linking CS chains with ECH in alkaline conditions.](image)

In the synthesis of semi-IPN and IPN hydrogels, the following parameters have been varied: cross-linking ratio, CS molar mass, and pH of the CS solution. Increasing the cross-linking ratio led to the increase of the gel fraction yield, this being in the range 89% - 92%, and to the decrease of the amount of CS removed from the semi-IPN gel after synthesis. The fraction of CS preserved in the semi-IPN composite cryogel increased also with the increase of the CS solution pH from 5 to 6.

Semi-IPN composite cryogels obtained with different cross-linking ratios (1/80, 1/60, 1/40 and 1/20) have been characterized by swelling kinetic in water. All semi-IPN composite cryogels displayed a super-fast swelling, the equilibrium of swelling being attained in 2 - 3 sec, the main difference between them being the values of the equilibrium swelling ratio which increased with the decrease of the cross-linking ratio from 1/20 to 1/80.

The images of the scanning electron microscopy (SEM) of the IPN composite cryogels (Figure 6) allowed to measure the average pore size, which was around 34 ± 5 μm in the case of IPN1.40.6, being two times lower than in the case of IPN1.60.6 (in these codes, the last figure shows the pH of the CS solution). Thus, a lower cross-linking ratio (1/60 compared to 1/40, samples IPN 1.40.6 and IPN 1.60.6) led to the increase of the pore size, and also to less compact and more accessible pore
walls (more permeable for diffusion of low molar mass species). SEM images in **Figure 6** show also the influence of CS molar mass on the gel morphology. As can be seen, the pore walls of the gel IPN2.60.6 synthesized with CS having \( M_v = 467 \) kDa are more compact than those of the gel IPN 1.60.6 synthesized with CS having \( M_v = 235 \) kDa.

**Figura 6.** SEM images of cryogels as a function of the cross-linking ratio and the CS molar mass, at a magnification of 1000x.

The potential for applications in separation processes has been evaluated by sorption/desorption experiments of a model dye, well known also as a drug, methylene blue (MB). Experimental isotherms have been described by some model isotherms (Langmuir, Freundlich și Sips) (**Figure 7A**), the experimental data being well described by Sips isotherm.

**Figure 7.** (A) Experimental isotherm of the MB sorption onto the cryogel IPN2.60.6 at \( pH = 5.5, 25^\circ C \), contact time 6 h, and curves resulted by non-linear fitting of three model isotherms. (B) Selective sorption of MB from a 1:1 mixture with methyl orange (MO); *left image*: cryogel before the contact with the mixture of MB and MO; *middle image*: cryogel after the selective sorption of MB; *right image*: crosssection in cryogel after the selective sorption of MB.

Separation of MB from its mixture with the anionic dye methyl orange (MO) has been followed by statical method. Initial solution was green due to the presence of both dyes, but was getting yellow after the selective sorption of MB onto the cryogel (**Figure 7B**). As **Figure 7B** shows, MB has been uniformly distributed inside the cryogel, this being also an indication for the uniform distribution of the negative centres able to bind the cationic dye. The selective sorption of cationic dyes recommend these composite cryogels as alternative for other sorbents with ion exchange properties and show their potential for applications in bioseparation or as drug delivery systems.

**Figure 8** presents the sorption kinetic of \( Cu^{2+} \) ions onto the IPN1.60 and IPN1.60 cryogels. As **Figure 8** shows, the equilibrium of sorption was establised within ~ 90 min, for both cryogels. The
experimental kinetic data have been fitted with two kinetic models by the non-linear regression method: pseudo-first-order kinetic model (PFO) and pseudo-second-order kinetic model (PSO). It was found that the PSO kinetic model described better the sorption of Cu\(^{2+}\) ions, and this demonstrated that the sorption mechanism was mainly chemisorption.

![Figure 8](image-url)

**Figure 8.** Sorption kinetic of Cu\(^{2+}\) ions onto the composite cryogels synthesized with CS1 (IPN1.60) and CS2 (IPN2.60); temperature 25 °C, sorbent dose 0.01 g, initial concentration of Cu\(^{2+}\) 444.5 mg/L.

**Figure 8** shows also the effect of the Cu\(^{2+}\) binding on the size of the cryogel, which collapsed but at a much lower level than the conventional hydrogels (results not included in this scientific report).

### 2.2. Synthesis and characterization of semi-IPN composite cryogels based on PAAm and a polyanion derived from potato starch

Another type of ionic composite cryogel was obtained by the cross-linking polymerization of AAm and in the presence of a polyanion (PA) prepared by the hydrolysis of of the copolymer PAN-g-potato starch (PS). The properties of cryogels having PA entrapped in the PAAm matrix were compared with those of the gels having PS as entrapped polymer. The properties of the composite cryogels have been further modified by controlled hydrolysis in alkaline medium. **Figure 9** present the SEM images of PAAm/PA60.5 cryogel, before and after hydrolysis.

![Figure 9](image-url)

**Figure 9.** SEM images of the composite cryogel PAAm/PA 60.5 before (A) and after hydrolysis (B).
The fast response at the external stimuli of the „smart” hydrogels is the most important characteristic for their applications, and therefore numerous methods have been utilized to control their response rate. The deswelling/reswelling kinetics of the macroporous composite cryogels having the same cross-linking ratio (1/80) but different by the monomer concentration, in the system ethanol/water, are presented in Figure 10.

![Figure 10](image1)

**Figure 10.** Cineticile de colapsare/reumflare în sistem etanol/apă ale criogelurilor compozite C-PAAm/PA80.3 (A) și C-PAAm/PA80.5 (B).

As can be seen in Figure 10, the response rate at reswelling of the collapsed cryogels was very fast. Composite cryogels having a concentration of monomers of 3 wt.% reached the equilibrium of collapsed state in about 2-3 min, while the composite cryogels obtained from an initial concentration of monomers of 5 wt.% reached the equilibrium in about 6-7 min. The response rate at reswelling was very fast, about 30 sec, for both cryogels, being unchanged until the end of the 3rd cycle. It was thus demonstrated that the deswelling steps have been faster when the cryogel was coming from a lower concentration of monomers.

The ionic character of semi-IPN composite cryogels having the polyanion PA entrapped in the PAAm matrix recommend these gels as materials sensitive at the ionic strength of the environment. Therefore, for the investigation of the deswelling/reswelling kinetics in response to changes of the ionic strength, the composite cryogels C-PAAm/PA80.3H (Figure 11A) and C-PAAm/PA80.5H (Figure 11B) have been selected, the 1 M NaCl/pure water being considered as bad solvent/good solvent.

![Figure 11](image2)

**Figure 11.** Cineticile de colapsare/reumflare în sistem 1 M NaCl/apă ale criogelurilor compozite C-PAAm/PA80.3H (A) și C-PAAm/PA80.5H (B).
As Figure 11A shows, the composite cryogel C-PAAm/PA80.3H reached both the equilibrium collapsed state and the equilibrium swollen state in about 2 min. On the other hand, the composite cryogel C-PAAm/PA80.5H reached the equilibrium swollen state in 20 min, this step being slower that the deswelling step, which has been attained in 5 min. This behavior could be attributed to the slow diffusion of electrolyte ions from the collapsed gel when this is immersed in water. The slower response rate of the C-PAAm/PA80.5H composite cryogel at reswelling in water, compared with the C-PAAm/PA80.3H cryogel prepared from a lower initial monomer concentration (3%), can be attributed to the higher density of polymer-polymer contacts caused by the excess of Na\(^+\) counter-ions. The time necessary to attain the deswelling equilibrium was also influenced by the monomer concentration, being higher at a higher monomer concentration (5 min for C-PAAm/PA80.5H and 2 min for C-PAAm/PA80.3H). This difference can be attributed to the cohesional forces between the hydrated chains of the ionic gel, which increase with the increase of monomer concentration.

The results obtained solving this objective have been disseminated by publication in six articles in ISI indexed journals, as follows:


4. M.V. Dinu, M. Pradny, E.S. Dragan, J. Michalek, Comparative morphological and swelling studies of porous hydrogels based on poly(hydroxyethyl methacrylate) and chitosan designed by ice-templating process and porogen leaching, J. Polym. Res. 20(11) Art 285, 2013


6. E.S. Dragan, M.V. Dinu, Design, synthesis and interaction with Cu\(^{2+}\) ions of ice templated composite hydrogels, Res. J. Chem. Env. 17(10), 4-10, 2013.

**Objective #3:** Preparation by cryogelation technique of novel porous „smart” matrices with response at more external stimuli based on poly(acrylamide) (PAAm) as matrix and poly(N,N-dimethylaminoethyl methacrylate) (PDMAEM), polymer sensitive to pH and temperature, as entrapped polymer, for the immobilization of bioactive components

**3.1. Synthesis of PDMAEM with different molar masses and controlled charge density**

The first step in the preparation of the novel cryogels was the synthesis of PDMAEM with different molar masses and charge densities. The synthesis of PDMAEM was performed by radical polymerization with 2,2’-azo-bis(isobutironitril) (AIBN) as initiator, in toluene as solvent. Polymer has been recovered by precipitation with white spirit. Further purification was performed by dialysis against distilled water for three days, at least. The dilute aqueous solutions were concentrated by gentle heating under vacuum, and the polymer was recovered then by freeze drying with Martin Christ, ALPHA 1-2LD device (24 h, at -57 °C and 0.045 mbar). Part of the PDMAEM samples have been quaternized with benzyl chloride. The cationic polymers thus obtained can be considered statistical copolymers consisting of uncharged, N,N-dimethylaminoethyl methacrylate, units, and
positively charged, N-methacryloyloxyethyl-N-benzyl-N,N-dimethylammonium chloride units, their general chemical structure being shown in the following Scheme.

The general code of ionic polymers is QxPDMAEMy, where Qx means the quaternization degree, expressed as moles of quaternized units per 100 N,N-dimethylaminoethyl methacrylate units, and y is the molar mass of the pristine PDMAEM in kg mol\(^{-1}\) measured by size exclusion chromatography. All ionic polymers were extensively purified in two steps: first by precipitation with n-hexane, when the quaternization degree was lower than 50 mol %, or by acetone, for higher degrees of quaternization. After two days of drying under vacuum, at room temperature, the polymers were solved in water and further purified by dialysis against pure water, and finally freeze dried with Martin Christ, ALPHA 1-2LD device (24 h, at -57 °C and 0.045 mbar). Finally, the polymer samples as white powder have been further dried in vacuum, at room temperature (~ 20 °C), in the presence of P\(_2\)O\(_5\). Chemical composition of the ionic polymers was proved by elemental analysis (the content of chloride ions determined by potentiometric titration with 0.02 N AgNO\(_3\) aqueous solution) and by \(^1\)H-NMR.

**Table 1** presents some characteristics of the PDMAEM samples used as parents in the synthesis of QxPDMAEMy.

<table>
<thead>
<tr>
<th>PDMAEM</th>
<th>(M_n), kg mol(^{-1})</th>
<th>(M_n/M_n)</th>
<th>(L_c^a), nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDMAEM60</td>
<td>57</td>
<td>2</td>
<td>91</td>
</tr>
<tr>
<td>PDMAEM85</td>
<td>85</td>
<td>2</td>
<td>135</td>
</tr>
<tr>
<td>PDMAEM110</td>
<td>110</td>
<td>2.5</td>
<td>175</td>
</tr>
<tr>
<td>PDMAEM250</td>
<td>250</td>
<td>2.4</td>
<td>398</td>
</tr>
</tbody>
</table>

\(^a\) polymer contour length = polymerization degree \(\times 0.25\) (monomer unit length, nm).

The values of quaternization degree, \(\alpha\), of the QxPDMAEMy samples, evaluated from the content in Cl\(^-\) ions and \(^1\)H-NMR spectroscopy, are presented in **Table 2**.

<table>
<thead>
<tr>
<th>Polycation</th>
<th>(\alpha), mol %</th>
<th>Cl(^-)</th>
<th>(^1)H-NMR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q5PDMAEM60</td>
<td>4.5</td>
<td>5.6</td>
<td></td>
</tr>
</tbody>
</table>
As *Tables 1* and *2* show, four samples of PDMAEM, with molar masses in the range 60 - 250 kg mol⁻¹, and three copolymers QxPDMAEMy, with charge densities ≤ 50 mol %, have been synthesized.

### 3.2. Synthesis of semi-IPN macroporous hydrogels based on PAAm and PDMAEM

The synthesis of multicomponent macroporous hydrogels has been performed by cross-linking radical cryopolymerization of AAm with MBAAm in the presence of PDMAEM, when a semi-IPN hydrogel having PAAm as a matrix and PDMAEM as entrapped polymer resulted. The samples PDMAEM85 and PDMAEM250 (*Table 1*) have been selected for the preliminary syntheses, when the influence of the PDMAEM molar mass and of the polymer concentration on the gel fraction yield (GFY) and on the fraction of PDMAEM remained in the semi-IPN gels after the extraction of the nonreacted components have been investigated.

The concentration of PDMAEM has been 0.5 and 1 wt.%, and the cross-linking ratio 1/80 and 1/40, the obtained results being presented in *Table 3*. For the synthesis of cryogels having the cross-linking ratio of 1/80, the following components have been used: 5.9 g solution of PDMAEM, 1.1 mL bidistilled water, 0.4742 g AAm, 1 mL BAAm with a concentration of 0.33 g/25 mL, 1 mL solution of tetramethylethylenediamine (TEMED) with a concentration of 0.625 mL/25mL and 1 mL solution of APS with a concentration of 0.2 g/25 mL. For the synthesis of cryogels having the cross-linking ratio of 1/40, the following components have been used: 5.9 g solution of PDMAEM, 1.1 mL bidistilled water, 0.4868 g AAm, 1 mL BAAm with a concentration of 0.643 g/25 mL, 1 mL solution of TEMED, and 1 mL solution of APS. In the cross-linking copolymerization of AAm with BAAm, TEMED catalysed the decomposition of APS with generation of free radicals, which initiated the polymerization. As can be seen in *Table 3*, the GFY has been higher in the case of PDMAEM with the molar mass of 250 kg.mol⁻¹, and the fraction of polymer removed during the extraction of the gels in water after synthesis decreased with the increase of the cross-linking degree, the concentration of PDMAEM having a lower influence on these two characteristics.

### Table 3

<table>
<thead>
<tr>
<th>Cryogel</th>
<th>Solution of PDMAEM, wt.%</th>
<th>Molar ratio BAAm:AAm</th>
<th>GFY b, %</th>
<th>Fraction of PDMAEM removed, wt.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAAm/PDMAEM250.0.5</td>
<td>0.5</td>
<td>1/80</td>
<td>80</td>
<td>47.5</td>
</tr>
<tr>
<td>PAAm/PDMAEM250.0.5</td>
<td>0.5</td>
<td>1/40</td>
<td>85</td>
<td>33.6</td>
</tr>
<tr>
<td>PAAm/PDMAEM250.1</td>
<td>1</td>
<td>1/40</td>
<td>86</td>
<td>36.1</td>
</tr>
<tr>
<td>PAAm/PDMAEM85.1</td>
<td>1</td>
<td>1/40</td>
<td>82</td>
<td>63.3</td>
</tr>
<tr>
<td>PAAm/PDMAEM85.1LN a</td>
<td>1</td>
<td>1/40</td>
<td>76</td>
<td>10.4</td>
</tr>
</tbody>
</table>

a Pre-freezing the reaction mixture at -196 °C; b gel fraction yield.
Therefore, for the synthesis of cryogels having PDMAEM85 as entrapped polymer, the cross-linking ratio has been 1/40, and polymer concentration 1 wt.%. However, it was observed a strong influence of PDMAEM molar mass, which has been removed up to 63.3 wt.%, in the case of this molar mass. To increase the fraction of PDMAEM85 remained after extraction in the PAAm matrix, a pre-freezing of the reaction mixture in liquid nitrogen (-196 °C) was performed. The comparative data in Table 3 show that the pre-freezing of the reaction mixture had a positive effect on the fraction of polymer preserved in the semi-IPN cryogel, but led to the decrease of the GFY. To better understand the intimate correlation between these parameters, the study will be extended to other composite cryogels of the same category.

Results of swelling in water at pH = 6.0 of some samples of semi-IPN PAAm/PDMAEM cryogels are presented in Figure 12.

![Figure 12](image)

**Figure 12.** Swelling kinetics of semi-IPN cryogels having PDMAEM250 (left) or PDMAEM85 (right) as linear polymer entrapped in the PAAm matrix.

As Figure 12 shows, the molar mass of PDMAEM did not have a significant influence on the equilibrium swelling ratio of the cryogels, this being of 22 g/g and 23.5 g/g when PDMAEM250 and PDMAEM85 have been entrapped in the PAAm matrix, respectively, at a cross-linking ratio of 1/40. Decreasing the cross-linking ratio at 1/80 led to a dramatic increase of the equilibrium swelling ratio, this behavior being observed also for other multicomponent semi-IPN cryogels. Pre-freezing of the reaction mixture in liquid nitrogen in the case of PDMAEM85 caused a small decrease of the equilibrium swelling ratio but also the decrease of the time necessary to reach the equilibrium of swelling (Figure 12, right).

The components present in the cryogels PAAm/PDMAEM have been identified by FTIR spectroscopy. Figure 13 contains the spectra of semi-IPN hydrogels synthesized in the presence of PDMAEM60 as entrapped polymer in the PAAm matrix, at both +20 °C (conventional hydrogel), and -18 °C (cryogel), after four days of extraction with pure water. The presence of PDMAEM is supported by the peaks located at 1730 cm\(^{-1}\) corresponding to C=O in ester group, and at 2821 cm\(^{-1}\) și 2771 cm\(^{-1}\) assigned to the CH\(_3\) groups, which are visible in both the spectrum of conventional hydrogel (sample 4) and the spectrum of cryogel (sample 5). The picks located at 1663 cm\(^{-1}\) and 1320 cm\(^{-1}\) have been attributed to the bending vibration of C-N bond in the amide groups in PAAm, and the peak at 1453 cm\(^{-1}\) has been attributed to CH\(_2\) groups.
Figure 13. FTIR spectra of the PAAm/PDMAEM hydrogels synthesized at 20 °C (sample 4, conventional hydrogel), and at -18 °C (sample 5, cryogel); sample 6 corresponds to cryogel treated with MCT-β-CD.
The synthesis of an IPN composite cryogel has been performed by postcross-linking of semi-IPN PAAm/PDMAEM cryogels with monochlorotriazynil-β-cyclodextrin (MCT-β-CD), which contains reactive groups capable to react with the tertiary amine groups in PDMAEM. FTIR spectrum of PAAm/PDMAEM cryogel treated with MCT-β-CD confirmed the presence of cyclodextrin by the peaks located at 1032 cm$^{-1}$ and 1082 cm$^{-1}$, which are attributed to the glycozidic ring.

Interior morphology of cryogel PAAm/PDMAEM, before and after the treatment with MCT-β-CD, is presented in the SEM images in **Figure 14**.

![Figure 14](image)

**Figure 14.** SEM images of cryogel PAAm/PDMAEM before (left) and after (right) the reaction with MCT-β-CD at a magnitude of 150x (up) and 500x (down).

As **Figure 14** shows, the cryogel morphology dramatically changed by the reaction with MCT-β-CD, the interconnected pores being visible after this step. Further investigations focused on this type of composite hydrogels are in progress following the preparation of novel controlled delivery systems for bioactive compounds (drugs, proteins).

**Project Director,**

Dr. Ecaterina Stela Dragan