Two of the project objectives were solved since January until December 2012, 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.**

The objective of this study was to construct cross-linked polyelectrolyte multilayers able to immobilize and release biological active compounds. For this purpose, poly(ethylene imine), a weak polycation ionized in a large pH range, two bifunctional cross-linkers, 3,3',4,4'-benzophenone tetracarboxylic dianhydride (BTCDA) and glutaric aldehyde (GA), different silica substrates and two enzymes, lysozyme (LYS) and pepsin (PEP), have been used. Single component cross-linked multilayer films based on linear poly(ethylene imine) [PEI(L)] and branched poly(ethylene imine) [PEI(B)] have been obtained by layer-by-layer assembly through BTCDA mediated hydrogen bonds and electrostatic interactions. The polycations were adsorbed from salt-free aqueous solutions onto: (i) Daisogel type silica microparticles, with diameter ranging between 40-60 μm and pores of about 100 nm; (ii) Davisil type silica microparticles, with diameter ranging between 9-11 μm and pores of 5-7 nm; (iii) silicon wafers with the surface of about 1 cm². The stabilization of PEI(L) and PEI(B) chains onto the silica surface was performed by cross-linking with BTCDA, which generated free carboxylic groups onto the solid surface. Then, the cross-linked layers, negatively charged in a large pH range, adsorbed a new polycation layer. Zeta potential measurements showed that the single component multilayer film increased regularly. Before enzyme immobilization, the third polyelectrolyte layer was cross-linked and activated with GA. After each modification step of the silica microparticles, streaming potential measurements have been performed. **Figure 1** shows the zeta potential curves as a function of pH for the PEI(B) multilayer films deposited onto Daisogel microparticles, before and after enzyme immobilization.
The alternate adsorption of polycation, cross-linking with BTCDA and GA, as well as enzyme immobilization onto single component cross-linked multilayer films introduced considerable amounts of carbon and nitrogen onto the solid silica surface. Therefore, the quantitative elemental analysis of the organic thin films after each modification step (adsorption/cross-linking/immobilization) was performed by X-ray photoelectron spectroscopy (XPS). The atomic ratios [C]:[Si] and [N]:[Si] showed that the films based on PEI(L) and PEI(B) increased linearly with the increase of the number of cross-linked polymer layers. After PEP and LYS immobilization onto the surface of the composite microparticles, the [C]:[Si] and [N]:[Si] atomic ratios showed that PEP was immobilized in a higher amount than LYS, which was attributed to the differences between the isoelectric points (iep) of the two enzymes. Therefore, the immobilization of PEP (iep ≈ 1) was favoured by the electrostatic interaction with the cross-linked multilayer film, while in the case LYS (iep ≈ 10), the immobilization process was disadvantaged by the electrostatic repulsions between it and the polycation layer.

The surface properties (average height, *h*ₐ, and average roughness, *R*ₐ) of the single component cross-linked multilayer films, obtained by atomic force microscopy (AFM), increased slightly after each modification step. These results have been correlated with those obtained by XPS analysis, in that the multilayer film with the highest amount of organic material deposited onto the Davisil microparticles had also the highest *h*ₐ and *R*ₐ values among all the films assembled onto silicon wafers (Figure 1).

This new type of single component multilayer films based on PEI can be used for the pre-coating of solid surfaces for the deposition of subsequent layers, for the immobilization of bioactive compounds or to construct surfaces with a fine balance between different types of interactions: hydrogen bonds, electrostatic and/or hydrophobic interactions. The immobilization of enzymes onto this type of multilayer thin films supports the possible application in the immobilization of biomolecules, construction of composite membranes, novel drug delivery systems etc.

**Objective 2: Design of novel multifunctional Porous Ionic Hydrogels (PIM) based on polyacrylamide and natural ionic polymers by cryogelation (IPN type ionic cryogels)**

Due to their capacity to mimic living tissues and respond to external stimuli, hydrogels have been used in medicine, as controlled drug delivery systems, tissue engineering, biosensors, mecanic actuators etc. For some applications, like artificial implants and controlled drug release systems, multicomponent hydrogels as semi-interpenetrating (semi-IPN) and fully-interpenetrating polymer networks (IPN) showed improved response rate to external stimuli and diffusion rate of bioactive compounds. Lately, an increased interest was devoted to macroporous hydrogels, due to their fast response rate to external stimuli comparing to conventional hydrogels.

For the synthesis of novel PIM hydrogels based on polyacrylamide (PAAm) and natural ionic polymers, the semi-IPN networks was firstly designed by cryogelation technique, and then chitosan (CS) and a anionically modified potato starch were used as natural polymers immobilized in the PAAm network.

**2.1. Synthesis and characterization of PAAm and CS based composite semi-IPNs and IPNs**

New semi-IPN type composite ionic hydrogels based on PAAm and CS have been obtained by cryogelation technique. IPN type composite ionic hydrogels based on PAAm and CS have been obtained by cross-linking of CS chains with epichlorohydrin (ECH), under alkaline conditions (Figure 2).
Figure 2. IPN type network formation, having two oppositely charged polymer networks, resulted by cross-linking of CS in alkaline conditions.

In basic media, the partial hydrolysis of amide groups in the PAAm matrix occurred, therefore, the cross-linking was accompanied by the simultaneous generation of some carboxylate groups. For the formation of semi-IPN and IPN networks, the following parameters have been varied: the cross-linker ratio, CS molar mass and the pH of CS solution. The gel fraction of the composite cryogels increased with the increase of cross-linker ratio, being ranged between 89% and 92%. The CS fraction removed from the composite cryogels decreased when the cross-linker ratio increased. The second parameter which contributed to the increase of CS fraction in the composite cryogel matrix was the pH of CS solution, the CS fraction removed from the semi-IPN network of the composite cryogel decreasing with the increase of pH from 5 to 6. The semi-IPN composite cryogels obtained using different cross-linker ratios (1/80, 1/60, 1/40 and 1/20) have been characterized by swelling kinetics in water. All semi-IPN composite cryogels presented a superfast swelling, the swelling equilibrium being reached in 2-3 s. The main difference consisted in equilibrium swelling ratios which increased with the decrease of cross-linker ratio from 1/20 to 1/80. Scanning electron microscopy (SEM) images of the IPN composite cryogels (Figure 3) showed that the average pore size was 34 ± 5 μm for the IPN1.40.6 network, being twice smaller than for the IPN1.60.6 network.

Figure 3. SEM images of the composite cryogels as a function of cross-linker ratio and CS molar mass, with a magnification of 1000x.

Therefore, the composite cryogels obtained using a smaller cross-linker ratio (1/60) had larger pores, but also less compact pore walls, which were more accessible for the diffusion of low molecular weight species. The influence of CS molar mass on the morphology of cryogel networks was also observed from the images presented in Figure 3. The IPN2.60.6 composite cryogel obtained using CS with $M_v = 467$ kDa showed more compact pore walls than the IPN1.60.6 composite cryogel obtained using CS with $M_v = 235$ kDa. The average thickness of pore walls, evaluated from SEM images, was approximately 4 - 6 μm and 12 - 14 μm for the IPN1.60.6 and IPN2.60.6 composite cryogels, respectively. Also, the dimensions of the cavities in the pore walls of IPN cryogels, evaluated using
image analysis software ACD Photo Editor v3.1, were 25 μm and 12 μm for IPN1.60.6 and IPN1.40.6 samples, respectively.

The potential applicability of the composite cryogels was evaluated by sorption/desorption of a model dye, methylene blue (MB), which also has medical properties. Experimental sorption data were fitted using Langmuir, Freundlich and Sips isotherm models (Figure 4A). The model isotherm which best fitted the experimental data was Sips isotherm. Separation of MB from a 1:1 mixture with the anionic dye, methyl orange (MO) has been investigated by the batch technique. Initially, the solution was green due to the presence of both dyes, but it became yellow after the sorption of MB by the cryogel (Figure 4B). MB was uniformly distributed inside the cryogel, indicating the uniform distribution of the active sites able to bind MB inside the cryogel.

![Figure 4](image)

**Figure 4.** (A) Experimental isotherm of MB sorption by IPN2.60.6 composite cryogel, at pH 5.5, 25 °C, contact duration 6h, and model isotherms obtained by non-linear fitting of three model isotherms. (B) Selective sorption of MB from a 1:1 mixture with MO; **left image:** the cryogel sample before the contact with the solution of MB and MO; **middle image:** the cryogel sample after selective sorption of MB; **right image:** the cross-section of the cryogel after selective sorption of MB.

In conclusion, the selective sorption capacity of model dyes recommend this type of cryogels as an alternative for other composite sorbents endowed with ion exchange properties, with potential applications in bioseparation or controlled drug delivery systems.

### 2.2. Synthesis and characterization of semi-IPN composite cryogels based on PAAm and anionically modified potato starch

A different type of ionic composite cryogels has been obtained by cross-linking polymerization of acrylamide with N,N’-methylenebisacrylamide in the presence of an anionic polyelectrolyte obtained by the hydrolysis of polyacrylonitrile chains grafted on potato starch (PA). The properties of the cryogels containing immobilized PA chains have been compared with those of the cryogels obtained using unmodified starch. Another route to change and control the properties of the composite cryogels was by performing some reactions of controlled hydrolysis. **Figure 5** shows the SEM images of PAAm/PA60.5 cryogel, before and after hydrolysis.
The fast response of “smart” hydrogels to external stimuli is the most important property for their application, therefore numerous methods have been used to control their response kinetics. In Figure 6 are shown the deswelling/reswelling kinetics in etanol/water mixture of the macroporous composite cryogels with the same cross-linking ratio of 1/80, but with different monomer concentration. As Figure 6 shows, the reswelling response rate of the collapsed cryogels was very fast. The composite cryogels with 3% monomer concentration attained the equilibrium collapsed state in 2-3 min, while the composite cryogels with 5% monomer concentration attained the equilibrium state in 6-7 min. The reswelling response rate was also very fast, of approximately 30 s for both cryogels, being unchanged until the end of the third cycle. It was demonstrated that at lower monomer concentrations, the deswelling steps were much faster then at high monomer concentrations.

The ionic character of the semi-IPN composite cryogels with anionic polyelectrolyte as entrapped polymer inside the PAAm matrix, recommends this type of gels as materials responsive to electrolytes. Therefore, the deswelling/reswelling kinetics of the composite cryogels C-PAAm/PA80.3H (Figure 7A) and C-PAAm/PA80.5H (Figure 7B) as a function of the modification of ionic strength has been investigated in 1M NaCl/water system.
As Figure 7B shows, the C-PAAm/PA80.5H composite cryogel attained the equilibrium reswelling in 20 min, this process being slower than the deswelling step, which was attained in just 5 min. This behavior was explained by the slow diffusion of electrolyte ions from the collapsed gel, when it was immersed in water. On the other side, the C-PAAm/PA80.3H composite cryogel (Figure 7A) attained the deswelling equilibrium, as well as the reswelling equilibrium, in 2 min. The slower response rate of the C-PAAm/PA80.5H composite cryogel at reswelling in water, compared with the C-PAAm/PA80.3H cryogel with lower 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.

Scientific papers published/sent in 2012 which mentioned the project as financing source:


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