GRAFTING OF POROUS POLYMERS FOR BIOLOGICAL APPLICATIONS

E.E. Smolko[1] , M. Grasselli[2]
[1] Comisión Nacional de Energía Atómica, Buenos Aires, Argentina
[2] Dpto. LaMaBio, Dpto. de Ciencia y Tecnología, Universidad Nacional de Quilmes, Bernal, Buenos Aires, Argentina

Abstract: Research on application of radiation processing to polymers is mainly focused by the National Atomic Commission (CNEA). The Agricultural and Industrial Applications Laboratory Unit operates at the Ezeiza Atomic Center since the end of 1980s. Since 1997 a new research group headed by Dr. O. Cascone and Dr. M. Grasselli, devoted to downstream processing of proteins from the University of Buenos Aires, was involved in the implementation of grafting techniques in collaboration with Dr. E. Smolko from CNEA. In 1999 Dr. M. Grasselli moved to the Universidad Nacional de Quilmes where he continued working on application of gamma radiation to materials for biotechnological process.

1. INTRODUCTION

The main idea of our research is to add new functionalities to polymeric membranes currently used in filtration processes. The addition of specific ligands to the internal surface of these materials gives them the particular retention properties of selected substances. In this case, the goal is the use of these new materials for the recovery of specific proteins from biological liquors. In the following years new specific applications on biological science were added.

Thus, gamma radiation from a Cobalt-60 source was applied to develop the following materials:

1. Grafting of commercial polysulfone and polyethersulfone membranes to obtain materials with specific adsorptive capacity to proteins.
2. Grafting of polyethylene membranes to obtain materials with a particular adsorptive capacity by molecular imprinting synthesis.
3. Grafting of synthesized HDPE to develop polymeric supports for bacteria immobilization applied to bio-catalysis process.
4. Radiosynthesis of porous polymethacrylate solids for analytical separation processes
5. Radiosynthesis of hydrogels confined into macroporous membranes to develop an extracorporeal bioartificial liver.
6. Simultaneous grafting of nanoporous track-etched membrane.

2. GRAFTING OF COMMERCIAL POLYSULFONE AND POLYETHERSULFONE MEMBRANES

Adsorptive membranes to recover specific proteins were developed by modifying commercial polymeric membranes by a simultaneous grafting technique.

The main monomer used for grafting was glycidyl methacrilate (GMA) because of its pendent reactive epoxy group, which can be modified in further chemical reactions, giving multiple possibilities to develop specific adsorptive properties to selected target molecules.

Grafting of macroporous membranes, in hollow fiber shapes, was developed using a low monomer concentration and a dose rate around 1 kGy/h. Porous materials expose a large area to the solvent, thus grafting yield (expressed in g %) is relatively high for the monomer solutions of low concentration. Additionally, the homopolymer formation is low and easily removed [1](Grasselli *et al*, 1999). The grafting was achieved in the internal surface, which has the advantage to not swell the membrane, keeping the initial shape. It is one of the main differences from grafted macroporous membranes achieved by pre-irradiation methods. Taking into account the low grafting yield, around 5 to 30 %, and the high surface areas of the trunk polymeric membranes (1 to 15 m^2/g, for different polymer shapes), membranes have coating polymethacrylate layers of thickness in the submicron range.

In order to analyze the adsorption behavior of these membranes to diverse proteins, different chemical ligands were immobilized onto the grafted membranes. Ligands range from small molecules electrostatically charged, such as the sulfonic groups [2], and the chelation groups such as iminodiacetic acid [3], and pseudo specific protein ligands such as triazinic dyes [4].

Changing the initial monomer concentration it is possible to control the density of ligand immobilised however grafting yields higher than 50 % do not improve the adsorption capacity to proteins [1]. On the other hand, copolymerization of different monomers has strong effects onto membrane properties and can be managed to alter the water permeation and adsorptive capacity.

For low hydrophilic ligands, such us triazinic dyes, major improvements in the specific protein adsorptive capacity were obtained by increasing the proportion of a hydrophilic monomer such as dimethyl acrylamide into the grafted copolymer [5,6]. In the case of highly hydrophilic ligands such as sulfonic groups, the addition of diethyleneglycol dimethacrylate to the copolymer reduces the swelling of the grafted layer and improves the flowing properties of macroporous membranes [7].

Grafted membranes were ensembled in a cartridge and were successfully applied to the purification of proteins of real samples such us milk, whey and calostrum. Recently adsorptive membrane technology was tested for the recovery of Lactoferrin from whey [8] and also Lysozyme from egg white with a very high productivity [7].

Highly specific adsorptive membranes can also be used for analytical proposes in the detection of desired proteins of medical interest. In this sense polysulfone macroporous flat membranes were also surface grafted using GMA. The protein Thioredoxin was immobilized by different chemical ligands (iminodiacetic acid and phenyl arsanilic acid) in the grafted membranes and discerns the protein orientation through specific enzymatic analysis and antibody recognition [9]. In a further step a *quimera* recombinant protein (Thiorredoxin-Glutamic acid decarboxilase) of clinical diagnosis interest, was successfully immobilized and stabilized in these modified materials [10].

3. GRAFTING OF POLYETHYLENE MEMBRANES TO OBTAIN MATERIALS WITH A PARTICULAR ADSORPTIVE CAPACITY BY MOLECULAR IMPRINTING SYNTHESIS

The technique of free radical polymerization with the addition a target molecule and monomers, rich “imprinted” polymers with specific adsorption properties for the chosen

molecule, is known as "molecular imprinting polymerization". Our goal was to combine this technique with surface grafting polymerization induced by gamma rays in order to produce adsorptive custom-made membranes.

Using as a target molecule a ternary complex of a peptide, a metal ion and vinyl pyridine, an imprinted polymeric membrane was achieved (see Fig 1). An imprinted layer to Bacitracin A was performed grafting with diethylenglycol dimethacrylate as crosslinker [11]. The material shows higher selectivity adsorption to Bacitracin than to similar molecules. Surface imprinting by radiation induced grafting onto membranes opens the possibility of improving selectivity of adsorptive materials.

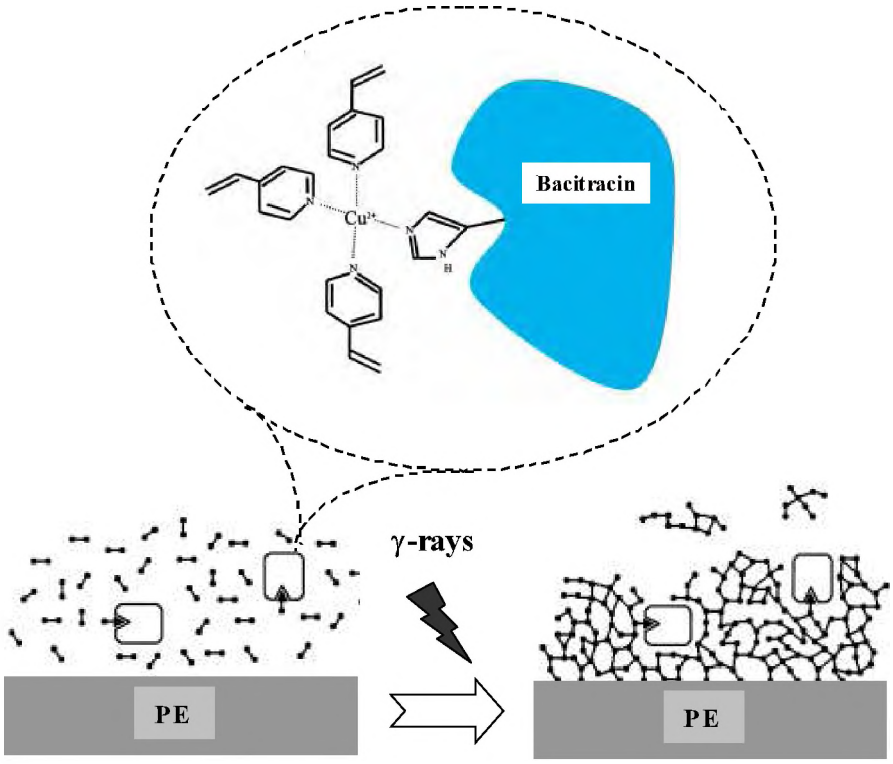

Fig. 1: Scheme of simultaneous imprinted surface grafting of PE by gamma rays in an aqueous solution

4. GRAFTING OF SINTHERIZED HDPE TO DEVELOP POLYMERIC SUPPORTS FOR BACTERIA IMMOBILIZATION APPLIED TO BIOCATALYSIS PROCESS

This method of polymers modification was recently extended to the obtainment of hydrogels supported on superporous open-cell polyethylene (average pore sizes from 60 to 200 microns) for bacteria immobilization [12] (Fig 2). PolyGMA surface grafted onto HDPE was hydrophilized by a ring opening reaction with ethylendiamine. Now, the modified HDPE is able to be loaded with bacteria in the micron-scale hydrogel that covers all the internal polymer surface (Fig 3).
This biocatalyst composed of grafted PE and bacteria was applied to increase the efficiency of catalytic processes with whole cells in the synthesis of nucleoside [13] and the production of

bacteriocins through fermentation of immobilized lactic bacteria (Britos and Grasselli, unpublished results).

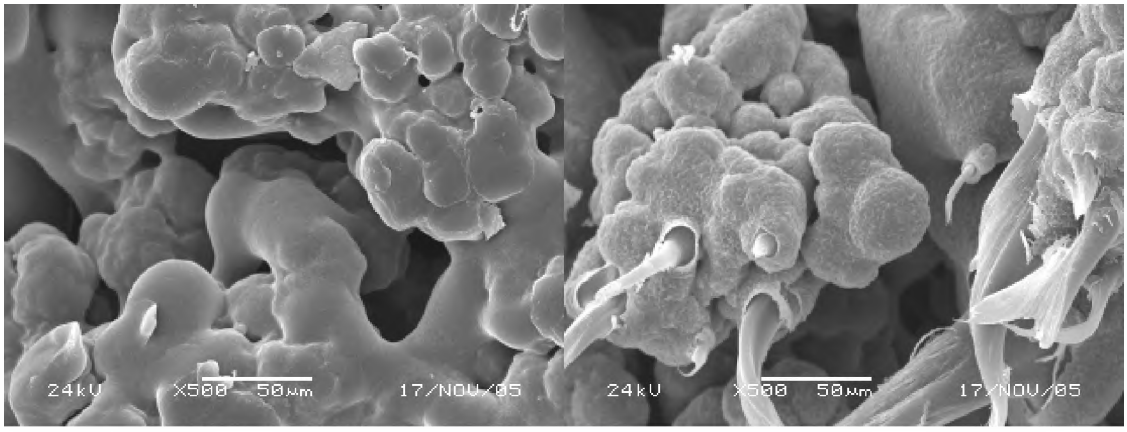

Fig. 2: SEM pictures of macroporous PE and GMA grafted PE

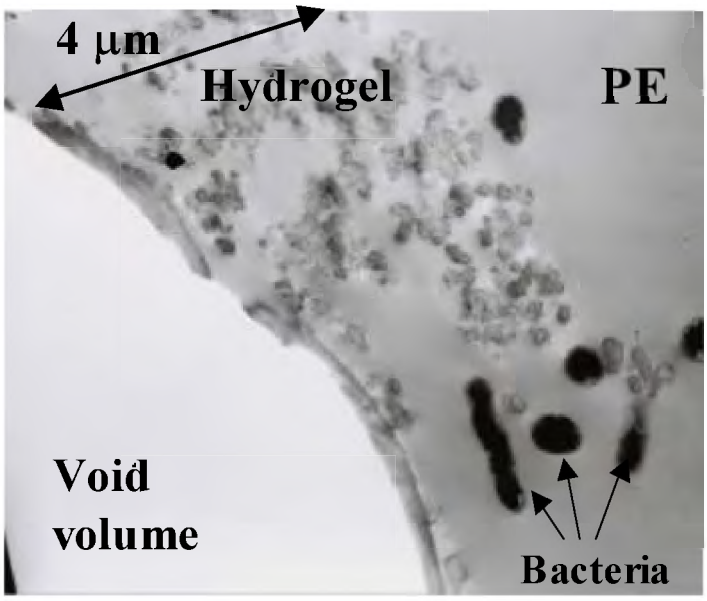

Fig. 3: TEM picture of immobilized bacteria onto macroporous grafted PE (12000x)

5. RADIOSYNTHESIS OF POROUS POLYMETHACRYLATE SOLIDS FOR ANALYTICAL SEPARATION PROCESSES

Other solid structures as polymeric microspheres and monolithic porous solids also were developed in collaboration with Dr. Agnes Zafrany, on the basis of the exposition of monomer solutions in polar solvents to ionizing radiation [14]. Synthesis of these macroporous polymers was the subject of an Argentine patent [15]. Its application to develop microextractors for capillary electrophoresis is being developed along with Dr. Vizioli in the Dept. of Analytical Chemistry of UBA [16, 17]. *In situ* radiosynthesis of porous monoliths and following ligand immobilization is a simple technique to have on-line microextractors. Specific peptides can be concentrated on-line and further released and analyzed by capillary electrophoresis. The main advantages of this technique are the simplicity of preparation, its homogeneity and no-frit requirement for the support retention into the column.

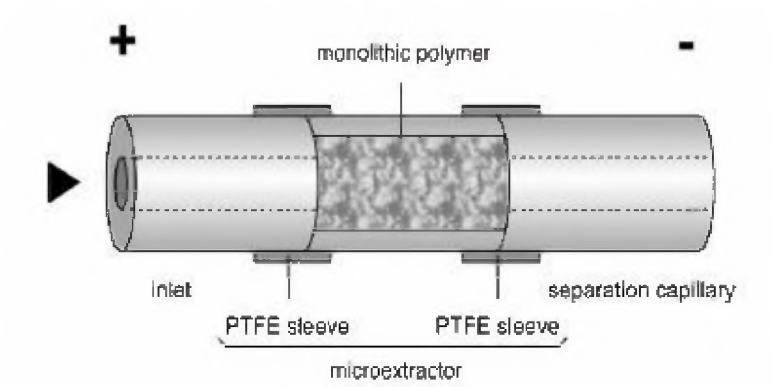

Fig. 4: Schematic representation of the monolitic microextractor into a capillary electrophoretic column

6. RADIOSYNTHESIS OF HYDROGELS CONFINED INTO MACROPOROUS MEMBRANES TO DEVELOP AN EXTRACORPOREAL BIOARTIFICIAL LIVER

Hydrogels have been used for many years to confine mammalian cells to be used in different prototypes of artificial organs. However it lacks enough mechanical resistance for many applications. Together with the Institute of Experimental Medicine of the Italian Hospital of Buenos Aires we are involved in the development of an extracorporeal bioartificial liver, based on a module of a hollow fiber membrane reactor. Our approach was to combine the intrinsic advantage of this module (high cell loading, compactness, etc.) with the biocompatibility properties of hydrogels.

The high energy and penetration of gamma radiation allows you to generate free radicals and induce polymerisation of monomers even in the void volume of porous structures. Thus, it is possible to prepare hydrogels by radiation-initiate polymerization of a monomer solution confined into a macroporous structure of a hollow fiber membrane reactor giving homogeneous embedded hydrogels [18].

We are currently measuring the mass transfer properties (to different solutes, gases and macromolecules of clinical interest) of the synthesized confined hydrogels to have bioprocess parameters, which could be able to design a prototype.

7. SIMULTANEOUS GRAFTING OF NANOPOROUS TRACK-ETCHED MEMBRANE

Swift heavy ions bombardment is a well-established technique to produce micro and nanopores onto polymeric films. However, new challenges are being focused to the introduction of functionalities through chemical wall modifications. One promising technique is the use of radical remnants of the traces to induce polymerization; in this way Mazzei and col., are developing this strategy [19].

Given the excellent results in our laboratory in surface modification of polymers by a simultaneous grafting technique induced by the gamma radiation, were conducted preliminary studies on their possible application to the modification of nanopores. Our group showed the first results on controlled nano-scale modification by this technique recently [20].

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Working Document