IMPRINTED POLYMER PEARLS FOR MOLECULAR RECOGNITION OF DIOSGENIN♦

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Abstract: The target of this study was the elaboration of a procedure for diosgenin molecularly imprinted polymers obtaining. The phase inversion method was used and it was aimed to prepare polymer pearls. In order to achieve the molecularly imprinted structures, copolymers of acrylonitrile with acrylic acid were synthesized by radical copolymerization in emulsion without emulsifier. The reaction was initiated by the redox system potassium persulfate – sodium metabisulfite.
The research studies allowed the assigning of the best parameters of the casting solution preparation, for the phase inversion and for the extraction.

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The rheological studies performed in dimethylformamide consolidated the choice upon the conditions for polymers solutions obtaining.
The rheological studies revealed changes of the polymer-polymer and polymer-solvent interactions and this could favor the molecular imprinting. These interactions depend on composition and concentration of the copolymer in solution, on concentration of diosgenine and on temperature. By the variation of the phase inversion and extraction conditions it was possible to obtain pearls molecularly imprinted with diosgenin.

Keywords: *polymer, pearls, molecular recognition, molecular imprinting, diosgenin, phase inversion, bioactive substances*

INTRODUCTION

A molecularly imprinted polymer (MIP) is a polymer that was formed in the presence of a molecule that is extracted afterwards, thus leaving behind complementary molecular cavities with specific electronic environment. The affinity for the target molecule suggests that they can be used in applications of advanced separations, biosensors, catalysts, the mechanism being similar to that of antibodies and enzymes.
Virtually two molecular imprinting methods exist, a chemical one and a physical-chemical one:

- polymerization
- phase inversion

The phase inversion method [1, 2] has the advantage that it begins from an already prepared polymer and that it allows the obtaining of spherical porous particles (0.5 – 1 mm diameter), resulting a material with a huge contact area. For phase inversion molecular imprinting, only some polymers or copolymers can be used, as for instance acrylonitrile – acrylic acid copolymers [3].
The main problems that have to be solved in the phase inversion case are finding a good solvent, common for the matrix copolymer and for the imprint and finding an optimum composition for the coagulation bath, so that the imprint diffusion in the bath or the chemical alteration to take not place. In this case, the reticulation of the polymeric structure is unnecessary, because the template remains physically built-in the polymeric matrix.
Diosgenine is a steroid with estrogenic bioactivity [4] and it can reduce the cholesterol from blood. It can be transformed in pregnelonone and progesterone. It is extracted especially from dioscorea species. However the extracts are very complex mixtures, from which the separation by classic methods is very laborious. This is the reason for proposing the molecularly imprinted polymers, known for their high selectivity.
The present work presents the results concerning the obtaining of the copolymer acrylonitrile- acrylic acid (AN-AA) pearls, imprinted with diosgenin, for the advanced separation of the bioactive compound from phytoextracts.

MATERIALS AND METHODS

Materials

In the work acrylonitrile (AN) p.a., acrylic acid (AA) p.a., sodium metabisulfite (MS) p.a., potassium persulfate (PK) p.a., sulfuric acid, p.a., dimethylformamide (DMF), technical, isopropyl alcohol, technical, methanol, technical, and diosgenin p.a. were used.

Methods

In order to obtain molecularly imprinted polymers with diosgenin, by phase inversion, the next steps were followed:

The acrylic copolymer obtaining
This was achieved by radical copolymerization of the acrylonitril with acrylic acid, in aqueous environment, initiated with the redox system: potassium persulfate - sodium metabisulfite, acid pH obtained with sulfuric acid.
The recipe was (mass percent):

- monomers concentration: 15 %;
- potassium persulfate (PK) concentration (calculated to the monomers): 0.5%;
- sodium metabisulfite (MS) concentration (calculated to the monomers): 0.5%
- H_2SO_4 concentration (relative to the monomers): 0.3%
- Polymerization temperature: 45 °C
- Polymerization time: 90 min.

The ratio between monomers was 70:30 or 80:20 (gravimetric). The adding order of the substances in the reactor is: water, sulfuric acid, AN, AA and then MS. Nitrogen is sparged into the solution for 10 – 15 minutes to put away the oxygen, which is an AN polymerization inhibitor. Then PK solution was added, the flask was introduced in the thermostated bath and mixing started.

The casting solution for the pearls
The copolymers prepared above were used to cast DMF solutions, having 8, 10 and 12% concentration (copolymer in DMF) and 4 and 5% diosgenin concentration (calculated to polymers). The dissolution of the copolymer lasts 45 min and that of diosgenin lasts 15 min, both at 70 – 75°C.

Obtaining the pearls
The solution was absorbed in a syringe and then it was trickled in a coagulation bath consisting in a 30 : 70 DMF : water solution. The pearls were maintained 30 min in the coagulation bath (to stabilize the structure).

Extracting the template
In order to extract the diosgenin (the template molecule), the pearls were introduced in a polyamide bag and let for extraction with methanol in the Soxhlet apparatus for 25 cycles. The pearls are maintained in distillated water until using.

RESULTS AND DISCUSSIONS

In order to analyze and compare the obtained solutions, the study of their rheological behavior was used as investigating method. Two types of copolymers were included in the study and tree different concentration of copolymer in DMF and two different concentration of diosgenin were tried. The influence of these three parameters is shown below.

In Figure 1, one can see the rheological behavior for two polymer solutions, prepared with the copolymer AN : AA 70 : 30 (named copolymer 1), respectively 80 : 20 (named copolymer 2). The polymer concentration for both DMF solution was the same (10%), and also the bioactive compound concentration was the same (5% related to the copolymer). The copolymer 1, with a bigger content of AA, has the medium viscosity 12.5 Pa.s, while the copolymer 2 has the medium viscosity 2.5 Pa.s.

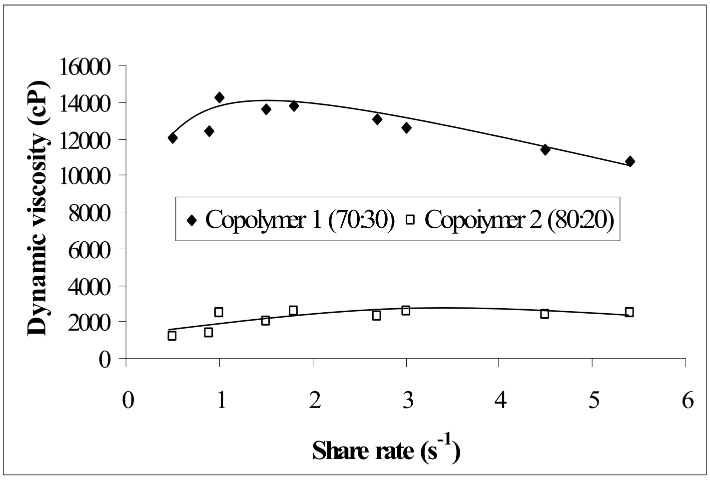

Figure 1. Rheological behavior at 25 °C of the solutions in DMF of the two copolymers (70AN:30AA and respectively 80AN:20AA), with 10% polymer concentration and 5% diosgenin (calculated to the polymer)

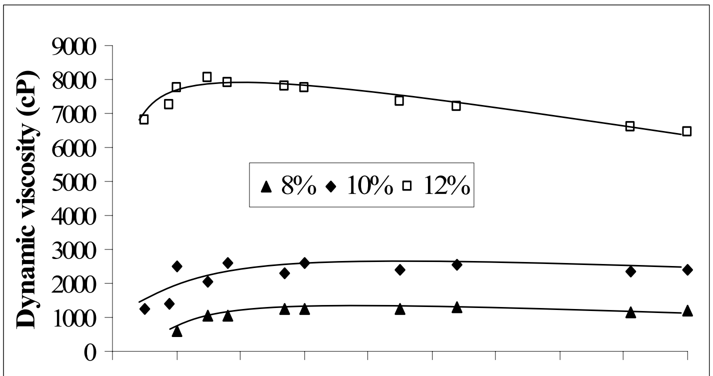

Figure 2. Rheological behavior at 25 °C of the DMF solutions of copolymer 2 (80AN:20AA), containing 5% diosgenin (calculated to the polymer) and different polymer concentrations (8, 10, 12%)

The copolymer 2 was chosen to be used for the next researches, concerning the influence of the polymer concentration of the DMF solutions. In Figure 2 it is represented the rheological behavior of three solutions of the copolymer 2, having various polymer concentrations (8, 10 and 12%). A 12% concentration makes the copolymer solution to be extremely viscous and very hard to extrude. Instead, the other two solutions of 8 and 10% concentration have reasonable dynamic viscosities and can be used in practice. They have had similar flow qualities and for this reason have been used for casting the polymer pearls.

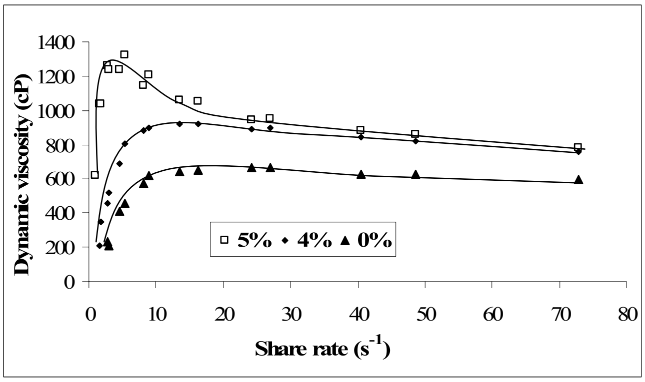

Figure 3. *Rheological behavior at 25°C of the DMF solutions of the copolymer 2 (80AN: 20AA), with 8% polymer concentration and different quantities of diosgenin (0, 4 and 5% - calculated to polymer)*

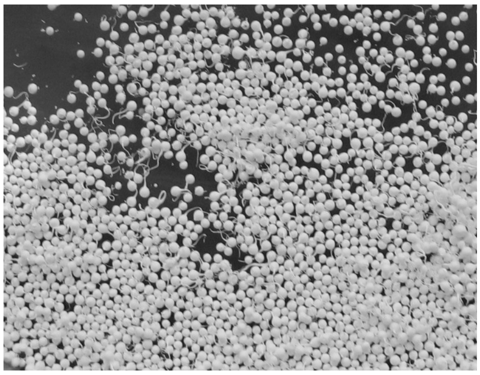

Figure 4. *Pearls obtained from DMF solution of the copolymer 2 (80AN: 20AA) with 10% polymer concentration and 4% diosgenin (calculated to polymer)*

Figure 3 describes the rheological behavior of the 8% copolymer solutions having various diosgenine concentrations (0, 4 or 5%, calculated to the polymer). One can see that by adding diosgenin in copolymer 2 solutions, the average dynamic viscosity is rising and the dilatant flow character is being amplified in the small share rates range. At big share rate range the solution behavior is light pseudo plastic, almost newtonian.
The Figure 4 shows the aspect of the pearls obtained from DMF solution of the copolymer 2, having 10% polymer concentration and 4% diosgenin (calculated to the polymer).

CONCLUSIONS

On the rheological behavior of the casting solutions for the obtaining of diosgenin molecularly imprinted pearls, an important influence has the copolymer concentration, while the diosgenin concentration has less influence.
Taking into account that the DMF solutions containing 8% and 10% copolymer, are characterized by quasi similar flow properties, the solution having a bigger concentration (10%) will be preferred, because it will involve a bigger diosgenin quantity, making the process more attractive from the practical point of view.
Diosgenin concentration of 4% is preferred for the casting concentrated solutions for pearls obtaining, because the rheological behavior of this solution recommends it as being more stable then the 5% diosgenin solution.

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