PROOFS FOR MOLECULAR IMPRINTING OF AN ACRYLIC COPOLYMER BY PHASE INVERSION

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Demonstrarea imprentării moleculare cu diosgenină prin inversie de fază a unor perle din copolimer acrilic a fost realizată atât prin analiza fazei lichide (extractele), cât şi prin analiza fazei solide (perlele). În acest scop, perlele, în diferite etape ale procesului de sinteză sau utilizare, au fost dizolvate în dimetilformamidă (DMF) şi au fost analizate prin cromatografie de lichide (HPLC) pe un aparat Agilent Technologies 1200 cu detector indice de refracţie (RID). Au fost obţinute semnale pentru copolimer, pentru diosgenină, dar şi pentru colesterol, un compus din aceeaşi clasă cu diosgenina, folosit pentru a demonstra selectivitatea noilor materiale faţă de molecula şablon.

The molecular imprinting with diosgenin by phase inversion of some acrylic copolymer pearls was proved both by analyzing the liquid phase (the extracts) and the solid phase (the pearls). For this purpose, the pearls at different stages of treatment were dissolved in dimethylformamide (DMF) and analyzed by liquid chromatography method (HPLC), working with an Agilent Technologies 1200 instrument with refractive index detector (RID). Signals were obtained for the copolymer, for diosgenin and also for cholesterol, a compound from the same class as diosgenin. The cholesterol was used to demonstrate the selectivity of the new materials towards the template molecule.

Keywords: imprinted polymer, phase inversion, molecular recognition, solid phase extraction, bioactive substances

1. Introduction

Molecularly imprinted polymers (MIPs) represent a new class of materials that contain artificially created receptor structures. A MIP is a polymer formed in the presence of a molecule that is extracted afterwards (figure 1), thus leaving complementary molecular cavities behind with specific electronic surroundings.

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The affinity for the target molecule suggests that they have the potential to significantly improve the performance of solid phase extraction, chiral separations, sensors, drug discovery and drug delivery processes, the mechanism being similar to antibodies and enzymes.

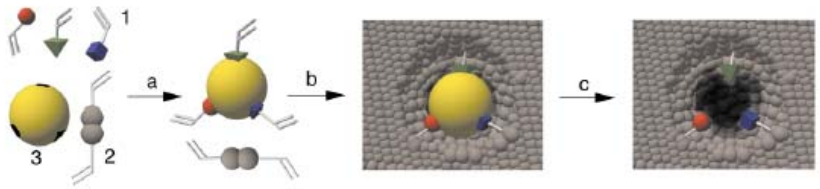

Fig. 1. An illustration of the molecular imprinting process of polymers

Since being pioneered by Wulff [1], molecular imprinting has become an effective way to prepare polymer materials that show a "memory effect" toward their templates and has been extensively studied by several groups [2, 3]. Molecular imprinting can also be considered as the selective manipulation of the shape, size and chemical functionality of a polymer matrix by a template molecule.

A wide range of chemical compounds have been imprinted successfully, ranging from small molecules such as drugs and herbicides to large proteins and cells. H-indole-3-acetic acid (IAA) is a plant hormone that exists in most plants and regulates growth and development in plants. Kugimiya and co-workers [4] have prepared IAA-imprinted polymer with *N*,*N*- dimethyl-aminoethyl methacrylate as the functional monomer in a non-polar solvent, chloroform. Also 9-Vinyladenine was synthesized as a novel functional monomer for molecular imprinting techniques [5]. Methacrylic acid (MAA) is the most widely used functional monomer in molecular imprinting methods. However, due to the weak hydrogen bonding interactions between MAA and some template molecules in polar solvents, the MAA based MIPs in polar solvents exhibited only very weak recognition [4] and in some cases no recognition at all [6].

The synthesis and processing capability of the nanoparticles with tailored structure and improved properties ensures tremendous perspectives for biomedical, environment and analytical applications: mimics for biological receptors [7], recognition of elements in sensors [8], stationary phases for chromatography [9] and solid phase extraction [10], micro extraction fibers [11], catalysis [12], molecularly imprinted membranes (MIM) [13-15], porous and imprinted membrane for dialysis [16]. The general principles, areas of applications and limitations of imprinted polymers have been extensively reviewed in the last years [17, 18].

Molecular imprinting by phase inversion approach means to incorporate a template molecule into a polymer matrix by phase inversion [19-22]. Removal of the template affords a cavity, which is complementary in size, shape and functionality to the template molecule. The phase inversion method has the advantage that it starts from an already prepared polymer. The main problems that have to be solved in this case are finding a good common solvent both for the matrix copolymer and for the imprint, as well as finding an optimum composition for the coagulation bath, so that the imprint diffusion in the bath or the chemical alteration would not take place.

The purpose of the paper is to present proofs of molecular imprinting of an acrylonitrile (AN) – acrylic acid (AA:AN) copolymer by the phase inversion method. The chosen substance to be imprinted in was diosgenin. Diosgenin, a steroid sapogenin, is the hydrolysis product by acids, strong bases, or enzymes of saponins. It has an estrogenic bioactivity [23] and it can reduce the cholesterol from blood. It is extracted especially from Dioscorea species. However the extracts are very complex mixtures, from which the separation by classical methods is very laborious. This is the reason for proposing the use of the molecularly imprinted polymers, known for their high selectivity. Also, it is anticipated that our current research will generate new fundamental knowledge about the molecular recognition phenomenon in artificial receptor systems. A number of industries could benefit from the commercialisation of the new developed technologies, particularly pharmacology, biotechnology, bioelectronics and environmental monitoring.

2. Experimental

2.1. Materials

The acrylonitrile (AN) : acrylic acid (AA:AN) copolymer was synthesized by radical copolymerization in aqueous environment, initiated with the redox system: potassium persulfate – sodium metabisulfite (PK – MS). The synthesis recipe was (mass percent): 12% AN, 3% AA, 0.5% potassium persulfate (PK), 0.5% sodium metabisulfite (MS), 0.3% H_2SO_4. For PK, MK, H_2SO_4 the concentrations were calculated with respect to the monomers.

2.2. Procedure

The polymerization temperature was 45°C and polymerization time: 90 min.

After drying and crumbling the copolymer, a solution of 10% copolymer in DMF containing 5 % diosgenin (calculated to polymer) was prepared by stirring the mixture at 70°C, during 60 min. The polymer pearls were obtained by dripping the copolymer solution with a syringe in a coagulation bath. The phase

inversion occurred at room temperature and water proved to be the proper nonsolvent.

In order to get the molecular imprinting, the template molecule (diosgenin) was extracted from the pearls with ethanol and chloroform in an ultrasonic bath during 60 minutes.

2.3. Analyzing methods

The molecular imprinting was confirmed by HPLC on liquid phase (the extracting solvent after the pearls extraction) and solid phase (after dissolution of pearls in DMF, in order to obtain a 1% solution).

HPLC analyses for the liquid phase performed on a Varian Pro Star instrument, with Inertsil ODS 3 C18 25 cm x 4.6 cm column, UV-Vis detector at λ = 193 nm, mobile phase acetonitrile 90% / water 10%, flow 1 mL/min and sample volume 25µL.

HPLC analyses for the solid phase performed on an Agilent Technologies 1200 series instrument with PLGel MiXED C column, refractive index detector (RID), using DMF as mobile phase and a 1 mL/min flow.

SEM images were also obtained and compared for pearls before and after extraction at two magnitude orders: 2000 and 5000.

3. Results

The obtained pearls have 0.6-1.2 mm in diameter after drying (figure 2b).

(a) (b)

Fig. 2. Pearls obtained from copolymer solution (80AN: 20AA) in DMF with 10% polymer concentration and 4% diosgenin (calculated to polymer). (a) in water; (b) after drying

The presence of diosgenin into the copolymer pearls after phase inversion was proved by analyzing the solvent after extraction by HPLC with UV-Vis detector. The extraction of diosgenin can be performed with ethanol and chloroform. Firstly the extraction of diosgenin was proved by comparing a HPLC

chromatogram for ethanol with the chromatogram of a 0.05% solution diosgenin in ethanol (figure 3).

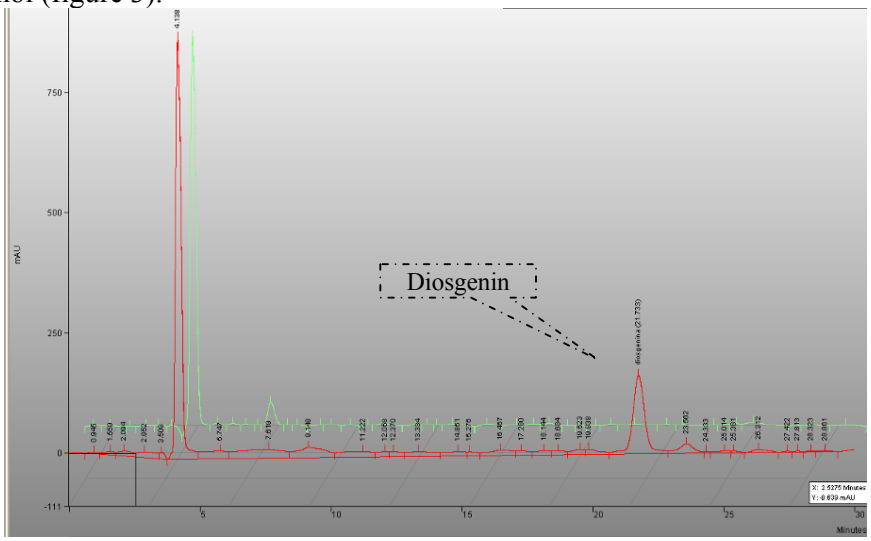

Fig. 3: HPLC chromatogram of ethanol (green) and of a 0.05% diosgenin solution in ethanol (red)

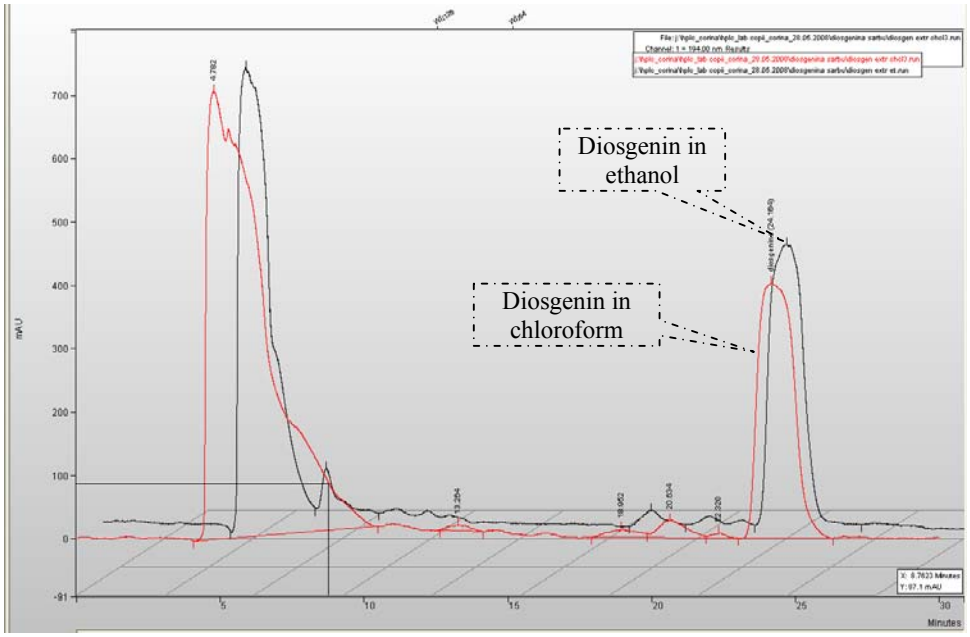

Fig. 4: HPLC chromatograms of the chloroform and ethanol solutions after the extraction of polymer pearls obtained from 10% DMF solution of copolymer and with 5% diosgenin (calculated to copolymer)

The demonstration for the pearls molecular imprinting with diosgenin was realized also by analyzing the **solid phase** (the copolymer pearls). For this purpose, the pearls at different stages of treatment were dissolved in dimethylformamide DMF and were analyzed by HPLC method. Firstly it was determinated the chromatogram for a copolymer – DMF solution. The copolymer has a retention time at 5.5 ± 0.2 minutes (figure 5).

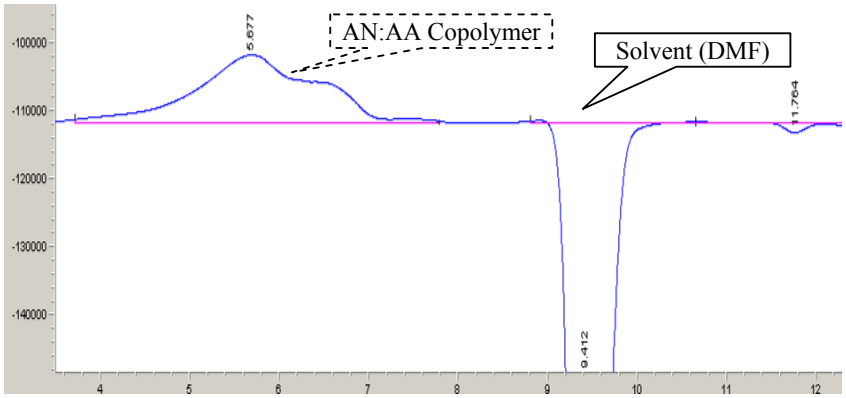

Fig. 5: HPLC chromatogram of a 1% copolymer solution in DMF

Secondly, the diosgenin signal was found at 10.6 ± 0.2 minutes by analyzing a solution of pearls imprinted with diosgenin, dissolved in DMF (1%) – figure 6.

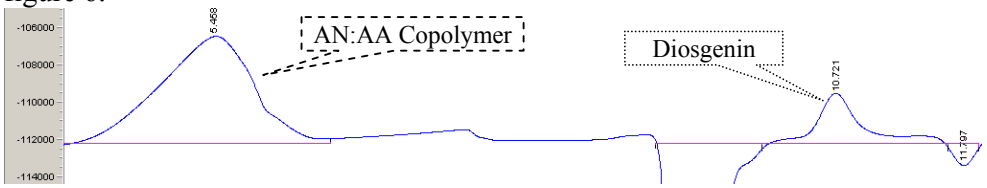

Fig. 6. HPLC chromatogram of a 1% diosgenin imprinted polymer solution

After the diosgenin extraction with ethanol in an ultrasonic bath during 60 minutes, the specific signal disappears (figure 7).

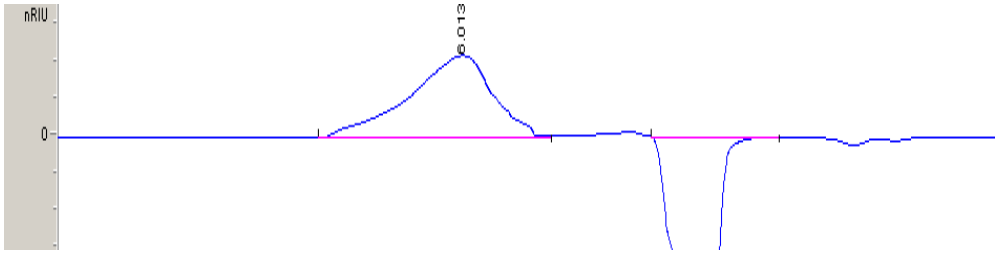

Fig. 7. HPLC chromatogram of a 1% imprinted polymer solution, after extraction

The solid phase extraction (SPE) capacity of the unimprinted pearls was also analyzed. The result was that diosgenin is low extracted from a prepared solution (figure 8).

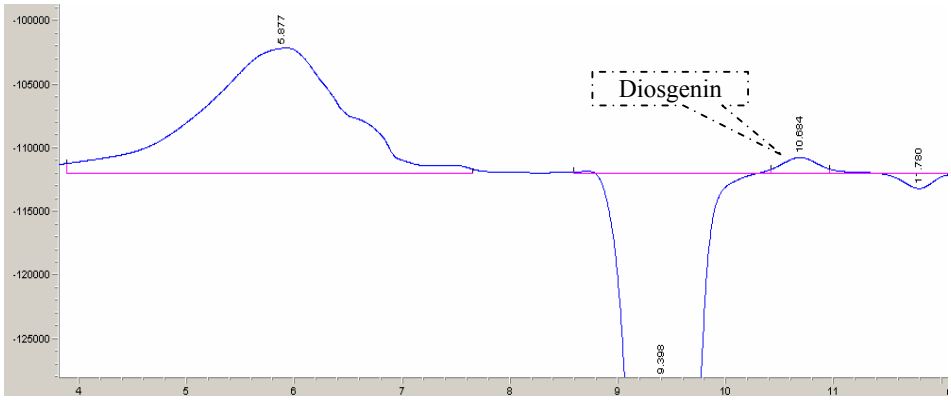

Fig. 8. HPLC chromatogram of a 1% unimprinted polymeric pearls solution

A compound from the same class as diosgenin, the cholesterol, was used to demonstrate the new materials selectivity towards the template molecule. In order to compare the sorption capacities of the two steroids upon the imprinted materials, the retention time and the corresponding area for a 0.1% steroids solution in DMF were determined (0.05% diosgenin and 0.05% cholesterol). Diosgenin was found again at 10.6 ± 0.2 minutes and cholesterol at 11.4 ± 0.2 minutes (figure 9).

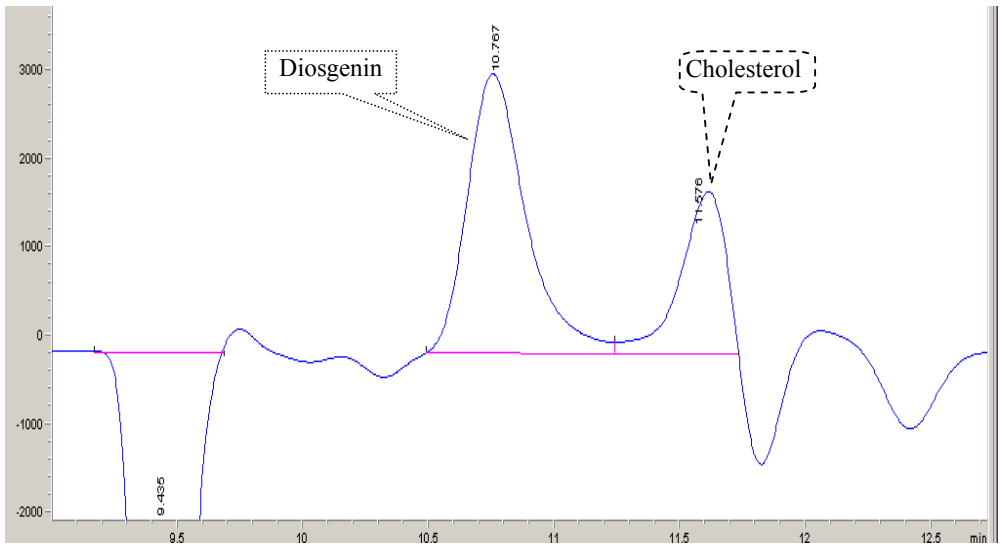

Fig. 9. HPLC chromatogram for 0.05% diosgenin and 0.05% cholesterol solution in ethanol

The SPE selectivity of the imprinted cavities for the template molecule is revealed by the fact that, from a diosgenine – cholesterol solution, only the diosgenine is well extracted (Figure 10).

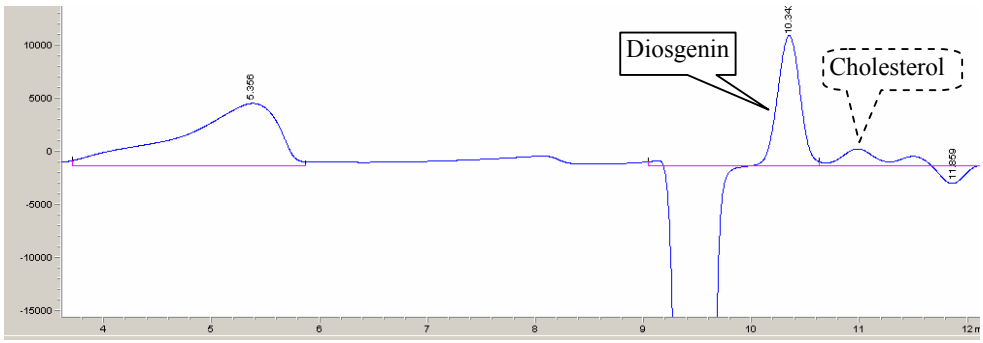

Fig. 10. HPLC chromatogram proving the selectivity of the molecular imprints towards the template molecule (diosgenin), by contacting with a solution 0.05% diosgenin and 0.05% cholesterol in ethanol

The next four figures are SEM images at two magnitude orders for the polymeric pearls before and after extraction of the diosgenin. The SEM images 11 and 13 are taken before extraction, while 12 and 14 after extraction.

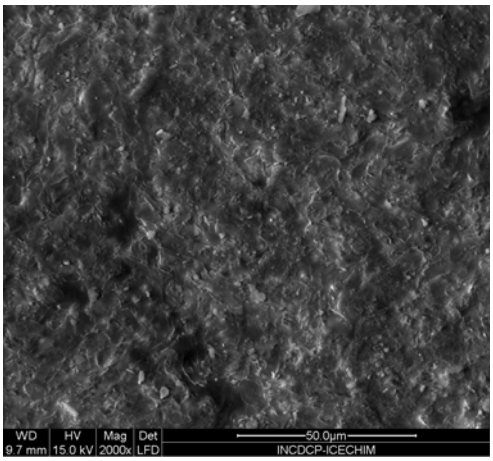

Fig. 11. SEM image of pearls before extraction, mag. 2000.

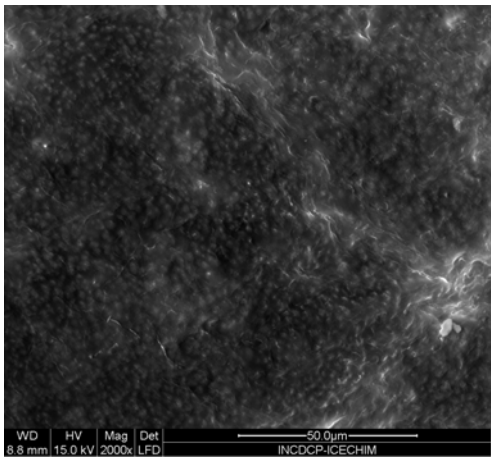

Fig. 12. SEM image of pearls after extraction, mag. 2000.

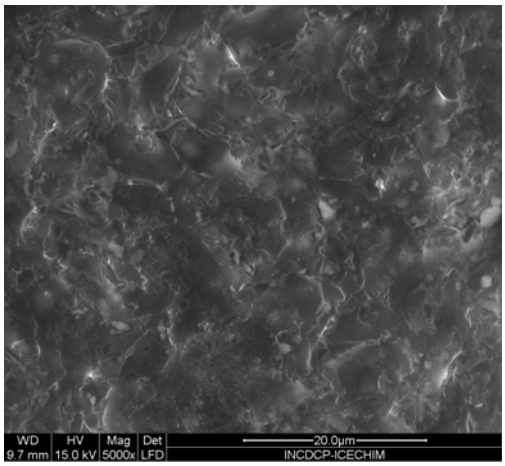

Fig. 13. SEM image of pearls before extraction, mag. 5000.

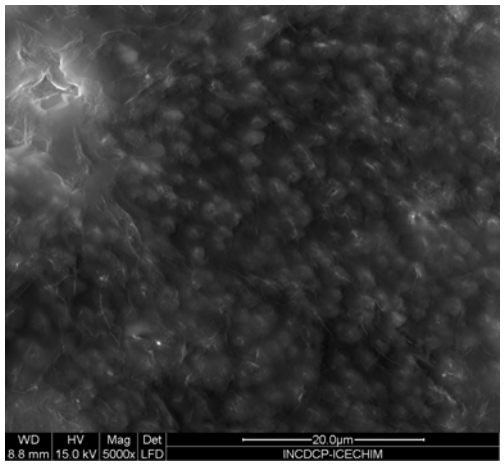

Fig. 14. SEM image of pearls after extraction, mag. 5000.

The pearls before diosgenin extraction with ethanol present whiter areas (figures 11 and 13) because diosgenin is white, while the pearls after extraction are more porous.

4. Conclusions

When the phase inversion and extraction conditions are properly chosen it is possible to obtain pearls molecularly imprinted with diosgenin.

Diosgenin was found in the extracts obtained with two solvents ethanol and chloroform, proving that it was introduced by phase inversion into the copolymeric pearls.

The molecular imprinting and the solid phase extraction selectivity were proved by HPLC on liquid and solid phases.

The solid phase extraction capacity of the unimprinted pearls was also analyzed showing that the diosgenin is low extracted from a prepared solution (diosgenin in ethanol).

The competition for the imprinted cavities between two similar bioactive substances (diosgenin and cholesterol) is improper because the molecular places are selective to the template (diosgenin). Thereby the molecular imprinting of an acrylic copolymer by phase inversion and the solid phase extraction selectivity of these new imprinted polymers was proved.

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