INFLUENCE OF CROSSLINKER/POROGEN RATIO UPON IMPRINTED POLYMER PARAMETERS

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Această lucrare tratează influența raportului reticulant/porogen asupra proprietăților particulelor polimerice amprentate molecular. Molecula țintă (analit) a fost acidul galic. S-au efectuat teste de sorbție/desorbție şi analize termice, microscopie electronică de baleiaj şi spectroscopie infraroşu. Procesele de adsorbție şi extracție au fost cuantificate utilizând cromatografia lichidă de înaltă performanță cu detector de indice de refracție.

This paper depicts the influence of the crosslinker/porogen ratio upon the properties of molecularly imprinted polymer particles. Gallic acid was used as target molecule (analyte). Sorption/recovery tests, and thermo-gravimetric analyses, scanning electron microscopy and infrared spectroscopy were performed. Adsorption and extraction processes were quantified using high pressure liquid chromatography with refractive index detector.

Keywords: imprinted polymer, recovery degree, solid phase extraction

1. Introduction

Molecularly imprinted polymers (MIPs) are materials that mimic biological sites and exhibit pre-determined selectivity toward a specific molecule (analyte, target molecule, template). During the last years MIPs have been used as synthetic materials with selective recognition ability for the target molecules in solid-phase extraction, sensors and chromatography [1]. The origin of molecular imprinting of inorganic and organic polymers goes back to Pauling's production of antibodies in vitro and Fischer's lock & key principle, which was for the first time

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described by Wuff et al. in 1972. Depending on the nature of the process, imprinting can be both chemical and physico-chemical. Chemical imprinting refers to a premix solution of a monomer-template complex preparation, followed by polymerisation and a crosslinking process, while physico-chemical imprinting implies phase inversion were the polymer solution is mixed with the template followed by precipitation in a non-solvent [2, 3].

Molecular imprinting can be: covalent, non-covalent (metal coordination bonding) and covalent-non-covalent mix, depending on the interaction nature between the functional monomer and the template. Although covalent imprinting is more specific than non-covalent imprinting, the first method was a major drawback in the small number of possible reversible covalent bonding with the target molecule. Thus the covalent technique is less used.

Many non-covalent methods for the preparation of MIP, such as bulk polymerization [4], suspension polymerization [5], emulsion polymerization, two-step polymerization, precipitation polymerization [6]**,** and sol-gel techniques were employed. Different MIPs structures, for instance irregular micro-particles and uniform nanoparticles [7], have been synthesized by conventional thermal/photo-polymerization, precipitation [8, 7] and micro-emulsion [9] polymerizations. MIP particles are generally packed into an HPLC (or other) column when used to separate desired target molecules, while thin films of MIPs, often coated on electrodes, are used for direct sensing of template molecules. Among these imprinting methods, bulk polymerization is the most used technique for particle preparation, due to its low cost production, high selectivity and reusability.

Polymers can be imprinted with a variety of bio-active molecules. Gallic acid is a well known natural antioxidant with potential protective role against oxidative damage diseases (coronary heart disease, stroke, and cancers) [10]. In this paper the influence of the ratio crosslinker/porogen upon the properties of MIP particles with gallic acid was investigated.

2. Experimental section

2.1. Materials

The monomer and solvents were purified before use, according to standard procedures. Ethyleneglycol dimethacrylate (EDMA) 98%, acrylic acid (AA), N,N-dimethylformamide (DMF) reagent grade and HPLC grade, ethanol 99.6% , acetonitrile reagent grade and 2,2'-azobis(2-isobutyronitrile) (AIBN) were purchased from Merck. Gallic acid (GA) with 99.8% purity and 10% crystallisation water was purchased from Fluka.

2.2. Equipment

Adsorption and extraction processes were assessed using high pressure liquid chromatography (HPLC 1200 Series with RID detector from Agilent

Technologies). A scanning electron microscope, Quanta 200, was used for microstructure analyses. Thermo-gravimetric analyses were performed on a TA Instruments Q5000IR. Infrared spectra were carried out on FTIR-Tensor 30 Brucker spectrometer with KBr pellets using 40 scans with 4 cm^{-1} resolution.

2.3. Preparation of Imprinted Polymer Particles

Three imprinted polymers, with various crosslinker /porogen ratios (MIP 1, MIP 11 and MIP 111) and a blank polymer (NIP 11) were prepared by bulk polymerisation. The recipes are given in Table 1. The reactions were carried out at 65^{o}C for 21 h. Selective sites were formed by non-covalent interaction between polar groups of gallic acid and polar groups of functional monomer (see Fig. 1). Analyte recognition was achieved by a similar non-covalent mechanism.

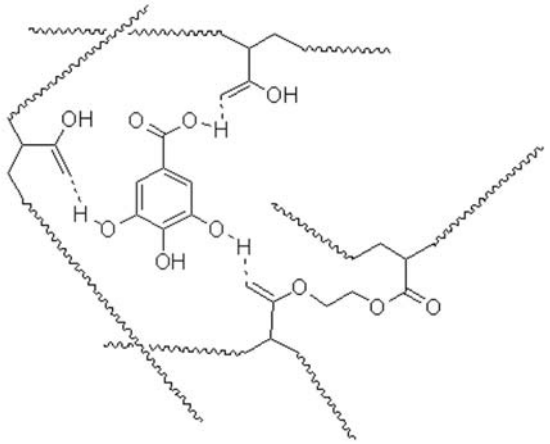

Fig. 1. Imprinted network formation

The functional monomer and acrylic acid were pre-mixed with gallic acid in order to establish connecting physical bonds (stage of self-assembly). Monomer – analyte solutions were introduced in polymerisation vials containing dimethylformamide as porogen solvent, ethylene glycol dimethacrylate as crosslinker, and 2,2'-azobis(2-isobutyronitrile) as radical initiator. The vials were then ultrasonicated for 10 minutes, purged with nitrogen for 5 minutes, and sealed.

Table 1

Recipes for the imprinted and blank polymers

Sample	AA (mmoles)	EDMA (mmoles)	DMF (mmoles)	GA (mmoles)	AIBN (mmoles)
MIP 1	3	15	52	0.5	0.183
MIP 11	3	10.6	52	0.5	0.183
MIP 111	3	15	64.6	0.5	0.183
NIP 11	3	10.6	52	-	0.183

The polymerisation vials were immersed in a heated water bath at 65^{o}C and held for 21 hours. After polymerisation the vials were broken and the polymers were mechanically grounded and sieved (70 μm fraction was further used). Extraction of gallic acid from imprinted particles was made by ultrasonication, using at least 3 portions of ethanol (5 ml of ethanol per 1g of MIP). Residual ethanol solutions (from filtration) were tested with 1% ferric chloride solution until there were clear (ferric chloride turns dark green in the presence of gallic acid). The extraction procedure should disrupt the polymer/template hydrogen bonding, so that the template can be washed away leaving behind the sculpted cavities.

2.4. Tests description

Adsorption tests

Adsorption tests consisted in placing 20 mg of imprinted polymers (MIPs) in 2 mL ethanol: acetonitrile = 85:15 (v/v) solution containing 2.257 g/Lgallic acid. For the absorption (recognition) of gallic acid by the active sites, the solvent has to enhance the GA affinity towards the polymer. That was why the chosen solvent for adsorption was a mixture of ethanol and acetonitrile, the latter being a non-solvent for gallic acid.

Recovery tests

10 mg Polymer samples containing gallic acid from previous adsorption test were submitted to extraction, with 1.5 mL ethanol by ultrasonication. A recovery degree was calculated.

Calculation method

Since the polymers resulted from the synthesis are insoluble, only the solutions remaining after adsorption or after recovery were tested by HPLC. The areas under each characteristic peaks, from elution diagram, are proportional to the concentration of each component in the analyzed mixture. The difference between the concentration of gallic acid in the remaining solution and initial concentration of gallic acid, in the reference solution, represents the adsorbed/recovered gallic acid concentration (per gram of polymer). All samples were tested under the same conditions: 25^{o}C, injected volume – 20 μL, elution flow - 1 mL/min, mobile phase – dimethylformamide (DMF).

The quantities of gallic acid adsorbed in the polymers were calculated with the equations (1) – (3), where: A_R and A_{rez} are the peak areas of gallic acid in the reference solution and in the remaining solution;, respectivelly; c_R and c_{rez} are the concentrations (g/L) of gallic acid in the reference solution (2.257g/L) and in the remaining solution; c_{ads} is the concentration of gallic acid (g/L) adsorbed by 0.02 g polymer; V_S the volume of the initial solution (0.02 L) and m_{GA} (g) is the

quantity of gallic acid adsorbed by 0.02 g polymer. The final quantities of gallic acid were presented as g GA/1g polymer.

$$c_{rez} = \frac{A_{rez} \cdot c_R}{A_R} \tag{1}$$

$$c_{ads} = c_R - c_{rez} \tag{2}$$

$$c_{ads} = \frac{m_{GA}}{V_S} \Rightarrow m_{GA} = c_{ads} \cdot V_S \tag{3}$$

Recovered gallic acid from polymers was calculated using equation (4) where: m_{rec} (g) was the recovered quantity of gallic acid; c_{rec}, was calculated using the calibration equation of gallic acid in ethanol; V^o_S, the initial ethanol volume (0.015 L).

$$m_{rec} = c_{rec} \cdot V_S^{\ o} \tag{4}$$

Calibration was performed in the range of 0.02-1% (or 0.16-8 g/l) gallic acid in ethanol. Calibration equation (with a correlation coefficient, r = 0.9984) is given by the regression equation (5) where: A represents the peak area of gallic acid, from ethanol solutions and c is the concentration (g/L) of gallic acid in the analyzed solution (in this case $c = c_{rec}$).

$$A = 1782800 \cdot c \tag{5}$$

Recovered gallic acid from polymers was expressed as g GA/1g MIP. Recovery degree of gallic acid, R, from imprinted polymers, was calculated, as the ratio between the recovered gallic acid and the adsorbed gallic acid (from previous test).

3. Results and discussions

Thermo-gravimetric analyses

The influence of gallic acid upon the polymers final properties was studied by thermo-gravimetric analysis (TGA) and performed on unextracted polymer samples, at 10 ^oC/min heating rate, under nitrogen.

Gallic acid is classified as a polyphenolic compound, which is well-known as inhibitor for polymerisation. Thereby, if such an inhibition effect had occurred, probably a change in the TGA curves could be noticed. The curves of decomposition for polymer MIP 11 and NIP 11 are given in Fig.2.

A sudden drop was noticed for MIP 11 polymer at about 50°C probably due to crystallization water loss from gallic acid. With this exception, both curve profiles remained the same through the whole degradation, indicating that gallic acid did not acted as an inhibitor of polymerization.

Thermo-gravimetric analyses were carried out for all MIPs; degradation profiles are given in Fig. 3, along with gallic acid. The highest thermal stability

was exhibited by MIP 11 polymer. Although MIP 1 and MIP 111 contained higher amounts of crosslinker, relative to the monomer (5:1 molar ratio), both MIPs showed lower decomposition temperatures. This may suggest lower curing degrees for MIP 1 and MP 111 polymers. Decomposition maxim for all MIPs were close; for MIP 1 and MIP 111 reached as high as 380 ^{o}C and 400 ^{o}C for MIP 11.

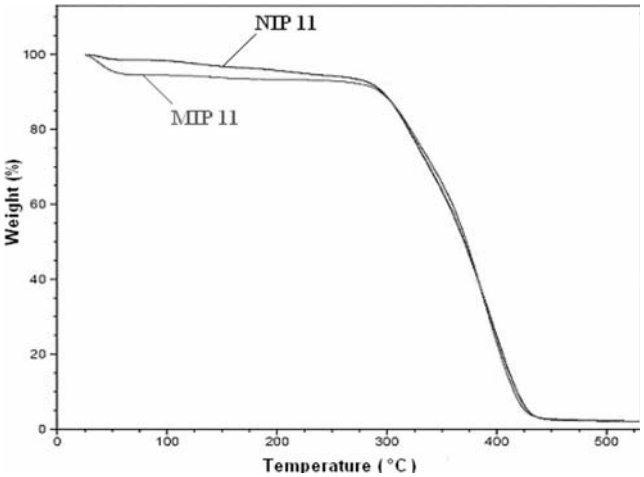

Fig.2. Thermo-gravimetric profiles of MIP 11 and NIP 11

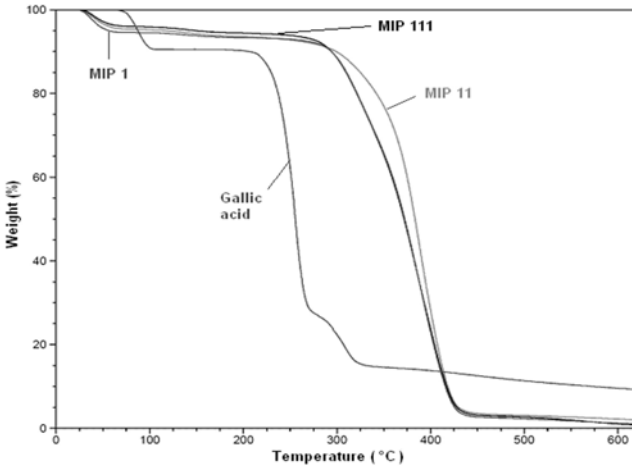

Fig.3. Thermo-gravimetric profiles of MIPs and gallic acid

Due to the fact that the solvent acts as conveyance environment for active species, similar to solution polymerization, the increase of solvent quantity may lead, in theory, to an increase in crosslinking degree (because the gel effect occurs later). With low solvent quantity, the gel effect occurs earlier, leading to low

crosslinking degrees and decreased thermal stability of polymers; therefore this being the case of MIP 1 and MIP 111.

Scanning electron microscopy

SEM micrographs of the three MIPs are shown in Fig. 4. It can be noticeD that MIP 11 shows a more compact structure (according to TGA this may be due to a higher crosslinking degree for MIP 11). The micrograph of MIP 111 and MIP 1 highlights the formation of several opened macropors. The presence of these open pores transforms the process of adsorption into absorption, but, further studies of porosity are to be reported in future paper. The high quantity of porogen in MIP 11 did not increase surface porosity, as it was expected. The crosslinking effect blocked out the internal pores or it diminished the pore channels towards the surfaces, leading to low communication with the imprinted sites.

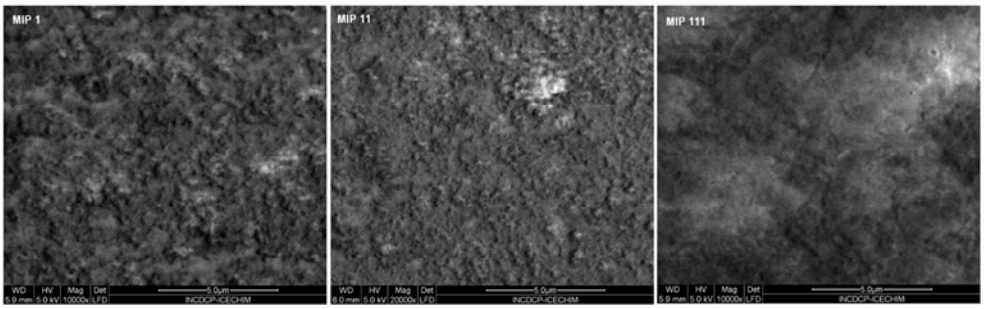

Fig.4. SEM images of MIP 1, MIP 11 and MIP 111

Infrared spectroscopy

The FT-IR spectra of blind sample NIP 11 and MIP 11 imprinted polymer are given together in Fig. 5, due to their obvious similarities. The C=C band at 1669 cm^{-1} from NIP 11 was more intense than the one from MIP 11, due to the presence of residual monomer in the former.

Table 2

Characteristic bands for MIPs, NIP 11 and gallic acid

Gallic acid (cm^{-1})	Group
3491	-OH (cryst. water)
3346	-OH (phenolic)
1694	-COOH
1242	-C-O
MIPs & NIP 11 (cm^{-1})	**Group**
2955	$-CH_3$ (as)
1722	-C=O
1660, 1669	-C=C-
1450	$-CH_3$(sym)
1250	-C-O or C-O-C

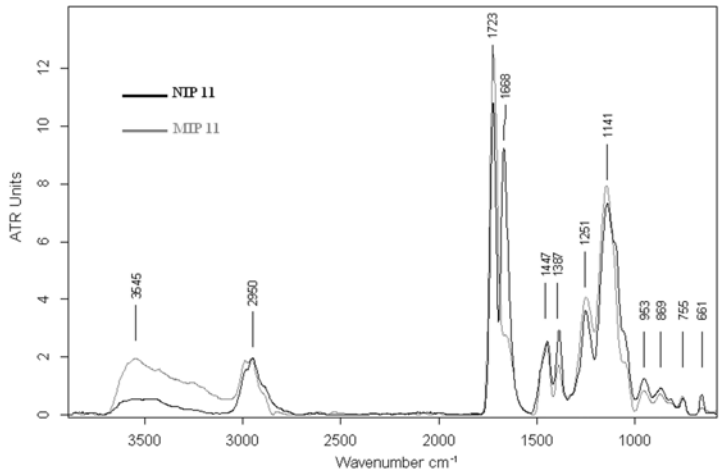

Fig. 5. FTIR spectra of NIP 11 and MIP 11 polymers

FTIR spectra of the imprinted MIPs are shown in Fig.6. The absence of characteristic bands of gallic acid, in all polymer spectra, confirmed the efficiency of the extraction method. The characteristic bands for gallic acid, MIPs and NIP 11 are summarized in Table 2.

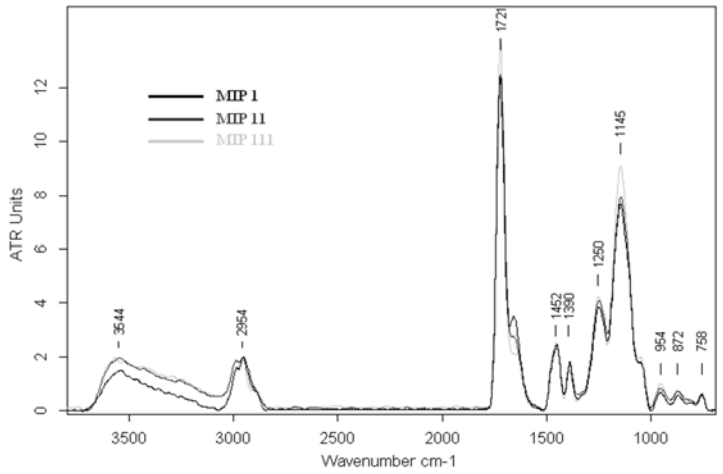

Fig. 6. FTIR spectra of MIPs

As mentioned in *section 2.3* the MIPs were extracted with ethanol causing probably the extraction of non reacted acrylic acid. Thus leading to a lower intensity of C=C band in all MIPs spectra. No supplementary bands appeared in the MIP 11 spectrum as compared to NIP 11, meaning that the gallic acid neither formed new covalent bonds with the polymerization parties, nor inhibited the polymerization process. This last fact was sustained by TGA results also.

Adsorption and recovery tests

The influence of crosslinker/porogen (C/P) ratio was highlighted by adsorption/recovery tests and sustained by thermo-gravimetric analyses (TGA), electronic microscopy (SEM) and infrared spectroscopy (FT-IR). Generally, the crosslinker and the porogen solvent have a significant contribution upon absorption and extraction processes. The crosslinking agent must ensure high stability, of the tailored cavities from MIP's, in the working solvent (at swelling), while the porogen must ensure the formation of opened pores which are responsible for communication with the possible internal cavities formed by synthesis. Subsequently, they both contribute to the transfer of the analyte inside and outside the MIP, if such porosity is formed.

Adsorption tests were performed using an ethanol: acetonitrile solution containing 2.257 g/l gallic acid. The up-take of gallic acid, m_{ads} (gGA/1gMIP), was calculated using high pressure liquid chromatography (HPLC) diagrams. The results are summarized in Table 3. After drying, the polymers were submitted to extraction tests. The recovered quantities of gallic acid, m_{rec} (gGA/1gMIP) and recovery degree, R_{GA} (m_{rec}/ m_{ads}), are given also in Table 3.

Table 3

The results of adsorption and recovery tests

Sample	MIP 1	MIP 11	MIP 111
m_{ads} (gGA/1gMIP)	0.007	0.0042	0.0047
m_{rec} (gGA/1gMIP)	0.0059	0.0028	0.0035
R_{GA}	0.84	0.67	0.75

MIP 1 exhibited the highest adsorption capacity for gallic acid and the highest recovery degree, due to an increased number of opened pores previously confirmed by SEM images. The fact that MIP 11 presents the lowest adsorption and recovery degree can be due to the polarity of the solvent. DMF is responsible for weakening the hydrogen bonding between gallic acid and monomer, leading to fewer active sites. This explains why the increase in porogen amount leads to lower specificity.

3. Conclusions

Thermo-gravimetric analyses, SEM, adsorption and recovery tests proved that higher molar C/P ratios (1/3.5 for MIP 1) may lead to relatively good parameters. In the case of MIP 11 imprinted polymer, a lower C/P ratio (1/4.9) led to higher crosslinking degrees and enhanced thermal stability, but on the other hand, it may lower the adsorption capacity and the recovery degree. FTIR analyses showed that gallic acid did not interact covalently with the polymerisation parties and could be extracted from the MIPs just by ultrasonication in ethanol. All the characterization methods were in good

agreement with the fact that 1/3.5 C/P molar ratio for imprinted MIP 1 was the optimum ratio for obtaining imprinted polymer particles with a high adsorption capacity and high recovery degree for gallic acid.

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R E F E R E N C E S

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