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DEVELOPMENT OF AMPHIPHILIC ADSORBENTS FOR THE STIMULATED UPTAKE AND RELEASE OF POLYPHENOLS

The development of amphiphilic molecularly imprinted polymers (MIPs) to target the stimulated uptake and release of polyphenols is studied, where MIP particles are synthetized through a free radical precipitation polymerization process. The formation of microparticles was evidenced by scanning electron microscopy. The adsorption capabilities of the materials synthetized were evaluated using solid phase extraction. The competitive adsorption of a mixture of polydatin + resveratrol + gallic acid was also studied.

Keywords: polyphenols, molecular imprinting, amphiphilic adsorbents.

Introduction. A polyphenol is an antioxidant chemical that prevents or neutralizes the damaging effects of free radicals. They provide a significant protection against the development of several chronic diseases such as cardiovascular diseases, cancer, diabetes, infections, aging, asthma, etc due to their anti-oxidant and anti-inflammatory activities. These active compounds are found in edible and non-edible plants. Indeed, they can be found in different vegetables and a growing activity concerning their efficient extraction, purification and concentration is observed nowadays [1]. The development of molecular imprinted materials for separation, concentration, purification of polyphenols, and also their control is investigated. In the imprinting process, the functional monomers are arranged around the template, then ”frozen” into position by polymerization with a high degree of cross-linking in the presence of a porogenic solvent. The obtained MIPs have the imprinted binding sites complementary to the shape, size, and functionality of the template and can thus specifically recognize the template molecule [2,3].

1. Synthesis of MIPs using Free Radical Polymerization. To synthesize the MIPs, a template in a monomer solution was added. The mixture was placed in a ultrasound bath during 30 minutes for promoting the interaction with monomer template (hydrogen bonds). After this we put the other components (cross-linker, initiator), and the mixture was purged with argon during 30 minutes. The final solution was placed in paraffin bath, previously heating at 60*℃*, and remained there for 24 hours. This synthesis was applied by a batch reactor. After synthesis, for a good characterization of particles, it is necessary to remove the template. With this goal, the particles were purified, using a large excess of MeOH/Acetic

Acid (9/1 v/v) followed by centrifugation. This process was repeated until the levels of the template became too low to detect. This step was monitored by a UV spectrophotometer. Finally, the particles were washed in large excess of MeOH for 24 hours. After removal of the MeOH, the particles were dried in a vacuum oven at 40*℃*.

Result and discussion. The surface morphology and the particle size of both MIP and Non-imprinted polymer (NIP) were analyzed using scanning electron microscopy (SEM). The analysis by SEM was in the microscopy center of University of Porto (CEMUP). Small particles were obtained (see Fig. 1), when synthetized by batch. The sizes of micro particles are about 1 μm.

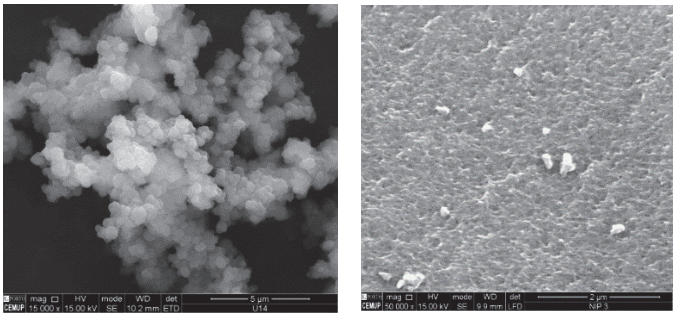

Fig. 1. SEM micrographs of MIPs and NIPs produced

2. Characterization of the Adsorption Capabilities for the Synthetized Molecular Imprint. An experimental procedure was used to perform the SPE evaluation of different particles concerning the uptake, release and separation of phenolic compounds. For this purpose, 6 cartridges, containing the particles, MIPs and NIPs, about 50 *mg* were proposed. These particles were conditioned with the same solvent that we used in the adsorption of the phenolic compounds, during 24 hours, and then the solvent was removed with the vacuum pump connected to the SPE system. The third step was washing, when we added 5 *ml* of solvent in the cartridge, the same solvent that we used in the loading step, then the solution was collected and analyzed in the UV spectrophotometer for verifying if there were phenolic compound release. The last step was elution where we added 5 *ml* of strong eluente (MeOH) with an objective to release the phenolic compounds that were still present in the particles. The solution collected was analized in the UV spectrophotometer.

Result and discussion. Fig. 2 presents the results obtained in these measurements (three replicas were performed). These observations seem to indicate a very small (or even no) molecular imprinting effect on the MIPs produced. In fact, adsorption in NIPs is often higher than in MIPs which is compatible with a non-specific interaction between the template and the particles.

These results (Fig. 3) are also consistent with the existence of non-specific interactions between different molecules and materials (a clear difference between MIP and NIP particles inside each class is not observed). Note that the increase of global adsorption should be a consequence of presence of water in the solvent, causing hydrophobic interactions.

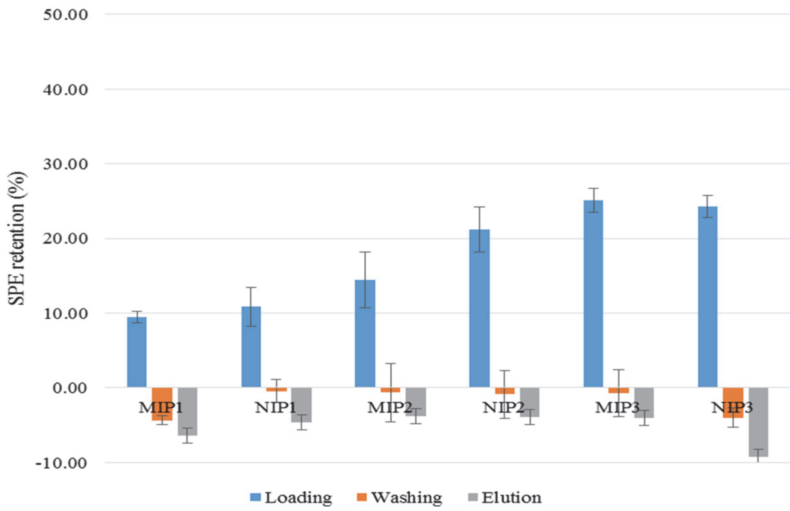

Fig. 2. SPE adsorption of polydatin in the synthetized MIP and NIP materials. ACN/MeOH 10/1 was used as the first solvent

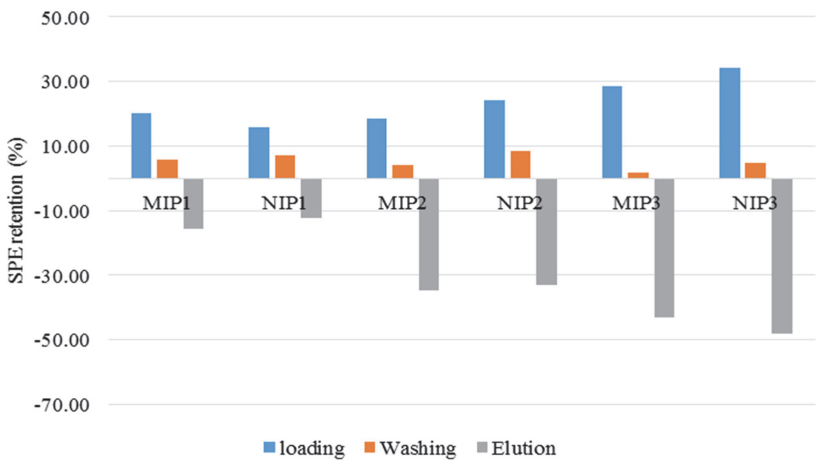

Fig. 3. SPE adsorption of a mixture containing gallic acid + polydatin + resveratrol on the synthetized MIP and NIP materials. ACN/H_2O 50/50 was used as the first solvent

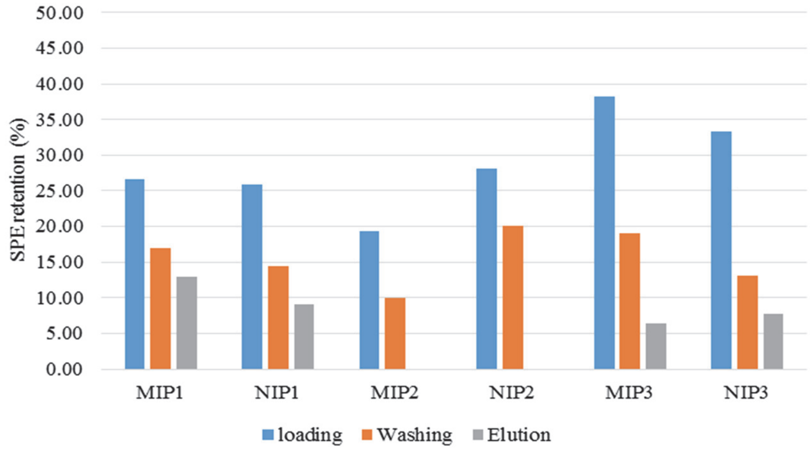

Fig. 4. SPE adsorption of a mixture containing gallic acid + polydatin + resveratrol on the synthetized MIP and NIP materials. ACN/MeOH 10/1 was used as the first solvent

In Fig. 4 the results for a similar experiment with the mixture gallic acid + polydatin + resveratrol but using ACN/MeOH 10/1 as a solvent are show. Note that, in spite of the non-specific retention observed, interesting outcomes are here evidenced, namely in comparison with the results of Fig. 2. Indeed, washing and elution steps seem to show a stronger interaction of one or two other molecules (gallic acid and/or resveratrol) with the materials when the testing is performed under the present conditions. Due to these results, the competitive adsorption of different molecules was studied in deeper detail, namely by performing the High performance liquid chromatographic (HPLC) analysis of the solutions after carrying out the SPE testing, as presented in the next sub-section.

3. Competitive SPE adsorption of polydatin+galic acid+resveratrol with HPLC analysis. Important limitations are found when detailed information on the adsorption/release processes are sought, especially when mixtures of molecules are considered, as briefly commented before. Thus, in order to get information concerning the competitive retention and release of polydatin, gallic acid and resveratrol in different classes of MIP/NIP particles synthetized (anionic, cationic, non-ionic), the solutions resulting from each SPE step were analyzed using HPLC. All the SPE competitive experiments were performed with the concentration of C=0.02 *mM* for each phenolic compound. Higher precision measurements are also expected with this technique (e.g. decreasing the high errors observed in the elution step). A very interesting result is presented in Fig. 5 Indeed, with MIP2, the total retention of gallic acid was observed in the SPE competitive process. This should be a consequence of the ionic interaction between gallic acid (anionic specie) and the cationic polymer network containing 2-(dimethylamino) ethylmethacrylate (DMAEMA).

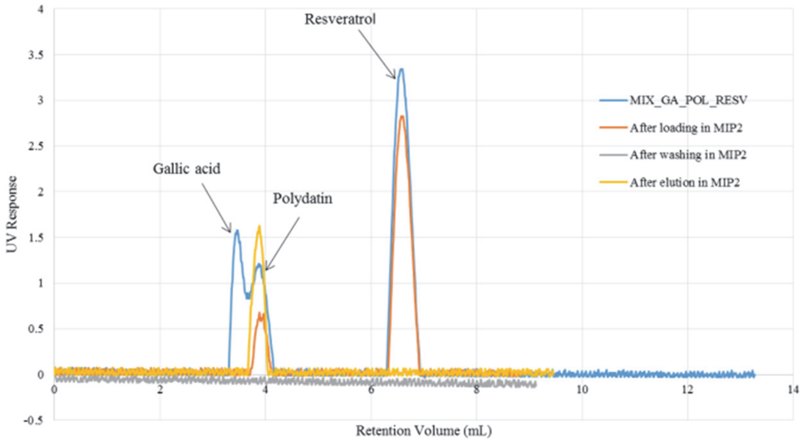

Fig. 5. HPLC analysis of the competitive SPE adsorption and release of the mixture polydatin+ gallic acid+resveratrol in MIP2. ACN/MeOH 10/1 was used as the first solvent

In Fig. 6 the global results for the competitive adsorption testing performed are presented. These bars indicate the percentage of retention for each compound in different materials and were calculated using the peak areas correspondent to the HPLC analysis. These results evidence again the high retention capability of MIP2/NIP2 for gallic acid retention with different solvents used in SPE.

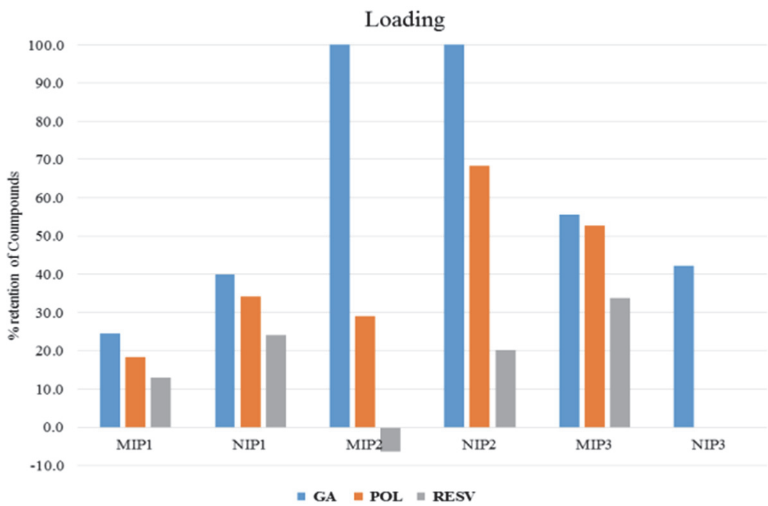

Fig. 6. Global results for the SPE competitive adsorption of gallic acid, polydatin and resveratrol in different materials

Conclusion. The results obtained, showed that the non-specific interactions between the molecules and the materials prevail under the conditions tested. Some selectivity of the materials towards the different molecules was observed but the most relevant result obtained was the ability of the materials based on DMAEMA to retain huge amounts of gallic acid. This capacity is due to the ionic interaction between the gallic acid (anionic specie) and the polymer network containing DMAEMA (cationic material). Hydrophobic interactions, namely in resveratrol retention, were also highlighted in these studies.

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ԱՄՖԻՖԻԼ ԱԴՍՈՐԲԵՆՏՆԵՐԻ ՄՇԱԿՈՒՄԸ՝ ՊՈԼԻՖԵՆՈԼՆԵՐԻ ԿԼԱՆՈՒՄԸ ԵՎ ԱՆՋԱՏՈՒՄԸ ԽԹԱՆԵԼՈՒ ՀԱՄԱՐ

Ուսումնասիրվել է ամֆիֆիլ մոլեկուլային դրոշմված պոլիմերների (ՄԴՊ) մշակումը՝ պոլիֆենոլների կլանումը և անջատումը խթանելու համար: ՄԴՊ-ի մասնիկները սինթեզվել են ազատ ռադիկալային պոլիմերացմամբ: Մասնիկների ձևավորումը հաստատվել է սկանավորող էլեկտրոնային մանրադիտակով: Սինթեզված նյութերի ադսորբման հատկությունները գնահատվել են պինդֆազային լուծահանումով: Ուսումնասիրվել է նաև պոլիդատին+ ռեսվերատրոլ+ գալաթթու խառնուրդի մրցակցային կլանումը:

Առանցքային բառեր. պոլիֆենոլներ, մոլեկուլային դրոշմում, ամֆիֆիլ ադսորբենտներ:

Г.Г. САДОЯН, Р.К. ДИАС, Н.Р. ОГАНЕСЯН

РАЗРАБОТКА АМФИФИЛЬНЫХ АДСОРБЕНТОВ ДЛЯ СТИМУЛЯЦИИ ПОГЛОЩЕНИЯ И ВЫСВОБОЖДЕНИЯ ПОЛИФЕНОЛОВ

Разработаны амфифильные молекулярно-импринтированные полимеры (МИП) для стимуляции поглощения и высвобождения полифенолов. Частицы МИП синтезированы осаждением свободнорадикальной полимеризацией. Формирование микрочастиц было подтверждено сканирующей электронной микроскопией. Адсорбционные свойства синтезированных материалов оценивали с использованием твердофазной экстракции. Была также изучена конкурентная адсорбция смеси полидатин + ресвератрол + галловая кислота.

Ключевые слова: полифенолы, молекулярный импринтинг, амфифильные адсорбенты.

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ՎԱՆԱՁՈՐԻ ՔԻՄԻԱԿԱՆ ԳՈՐԾԱՐԱՆԻ ՊՈՉԱՄԲԱՐՆԵՐԻ ԱՐՏԱՀՈՍՔԻ ՎՏԱՆԳԸ
(Վանաձոր)

Ուսումնասիրվել է Վանաձորի քիմիական կոբինատի պոչամբարների արտահոսքը ուժեղ երկրաշարժերի և սողանքների դեպքում և կատարվել է համապատասխան վերլուծություն: Պոչամբարների պատնեշի պատռման դեպքում, ցույց են տրված նաև պոչանքների հնարավոր ներթափանցման տարածքները և աղտոտման մասշտաբները:

Առանցքային բառեր. պոչամբարներ, տեխնածին վտանգ, արտահոսք, սողանք, ռիսկային գոտիներ, սեյսմաակտիվ գոտի:

Վանաձոր քաղաքը գտնվում է Հայաստանի ամենաբարձր սեյսմաակտիվ գոտիներից մեկի՝ Փամբակ-Սևանի ակտիվ բեկվածքի ազդեցության սահմաններում: Ըստ ՀՀ սեյսմիկ շրջանցման հավանականային քարտեզի՝ Վանաձոր քաղաքը գտնվում է այնպիսի սեյսմիկ վտանգի գոտու սահմաններում, որտեղ գրունտի սպասվող հորիզոնական արագացման մեծությունը կազմում է 0.5g (9-10 բալ):

Անդրադարձ է կատարվում, մասնավորապես, Վանաձորի քիմիական գործարանի առկա պոչամբարներից թունավոր նյութերի՝ որպես տեխնածին