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Controlled release of the herbicide simazine from computationally designed molecularly imprinted polymers

Elena V. Piletska*, Nicholas W. Turner, Anthony P. F. Turner, Sergey A. Piletsky

Institute of BioScience and Technology, Cranfield University, Silsoe, Bedfordshire, MK45 4DT, UK

Abstract

The present study describes the development of materials suitable for environmental control of algae. Molecularly imprinted polymers (MIPs) were used as simazine carriers able to provide the controlled release of simazine into water. Three polymers were designed using computational modelling. The selection of methacrylic acid (MA) and hydroxyethyl methacrylate (HEM) as functional monomers was based on results obtained using the Leapfrog^{TM} algorithm. A cross-linked polymer made without functional monomers was also prepared and tested as a control. The release of simazine from all three polymers was studied. It was shown that the presence of functional monomers is important for polymer affinity and for controlled release of herbicide. The speed of release of herbicide correlated with the calculated binding characteristics. The high-affinity MA-based polymer released ~2 % and the low-affinity HEM-based polymer released ~27 % of the template over 25 days. The kinetics of simazine release from HEM-based polymer show that total saturation of an aqueous environment could be achieved over a period of 3 weeks and this corresponds to the maximal simazine solubility in water. The possible use of these types of polymers in the field of controlled release is discussed.

Keywords: molecular imprinting, computational modelling, simazine, herbicide, controlled release

*Corresponding author. Tel: +44 (0)1525 863534, Fax: +44 (0)1525 863533
E-mail address: e.piletska@cranfield.ac.uk

1. Introduction

Simazine is one of the most popular photosynthesis-inhibiting herbicides. It is used in many countries to kill broad-leaved weeds and also to control vegetation and algae in farm ponds, fish hatcheries, swimming pools, fountains, ornamental fish ponds and water-recirculating cooling towers. Although the PAN Pesticide Database (http://www.pesticideinfo.org/Index.html) informs that in some concentrations simazine might be toxic to fish and aquatic ecosystems, simazine is generally considered as non-toxic for most species [1, 2]. Although EU directives have banned the use of simazine on non-cropped land, its use is still permitted on cropped land and in ornamental water (ponds, aquariums) [3]. According to available literature, simazine is effective at controlling unicellular and attached filamentous algae at a concentration of 0.1- 1 milligrams per litre (mg L^{-1}) [2, 4].

It is well known that it is extremely difficult to keep pond water in good condition. During the spring when the temperature goes above 10 ºC the water becomes green due to uncontrolled growth of different types of algae (Fig. 1). There are many commercial products which can be used for algae control and most of these products have simazine or 2-chloro-4,6-bis (ethylamino)-s-triazine as the only active ingredient. These products are available in liquid, tablet or powder form. It is recommended to add simazine products regularly to the water in order to keep it clear from filamentous (blanket weed) and unicellular algae. Unfortunately, simazine administration in this way is labour intensive, time-consuming and also has one important additional drawback in that it results in fluctuation of the simazine concentration. This could lead on the one hand to uncontrolled algae growth and on the other, irreversible damage to other organisms or whole ecosystems. The recommendations of the commercial producers tend to underestimate the working concentration of simazine and go for a maximum soluble level, which corresponds to 3.5- 5 mg L^{-1}. An innovative solution is required in order to find a user-friendly, simple and controlled method of simazine administration. The ideas presented in this

paper work toward this by the design of a specific molecularly imprinted polymer (MIP), which could release the template at required rate.

Molecular imprinting technology is known as a method of preparation of specific recognition sites by formation the complex between template and functional monomers [5]. The molecular complex between template and functional monomers is preserved using excess of polymerisable cross-linker. Thermal or photochemical initiated polymerization produces a highly cross-linked insoluble polymer. The extraction of the template from the MIP creates cavities in the matrix, which are complementary in both shape and chemical functionality to those of the template. Traditional fields of MIPs application include separation [6-8], synthesis and catalysis [9, 10] and sensors [11, 12]. These applications are mainly based on the selective adsorption characteristics of molecular imprinted polymers.

The application of desorption properties of MIPs is a relatively new area. Early studies delivered promising results that showed that these affinity materials could be used for controlled delivery of drugs [13-15]. Although molecular imprinting technology has a potential for creating custom-made carriers for variety of chemicals and biomolecules, intensive development and optimisation is necessary in order to bring the controlled release application into practice. Among the features, which should be included in a "dial-the-MIP" protocol, is the rational selection of the functional monomers, polymer format and increasing the polymer capacity.

In this paper, a feasibility study on the possibility of using a simazine-specific molecularly imprinted polymer for controlled release of simazine into water is described. As far as we know, it is the first report on the application of MIPs for sustained release of herbicides into the environment.

2. Material and methods

2.1. Materials

Acetonitrile, dimethyl formamide (DMF), water (all HPLC grade), ethylene glycol dimethacrylate (EGDMA), 1,1-azobis (cyclohexanecarbonitrile), MA were purchased from Sigma (UK). HEM was purchased from Aldrich (UK). Simazine was purchased from Riedel-de Haën (Fluka, UK).

2.2. Molecular modelling of monomer-template interactions

In order to simulate the monomers/template interactions a Silicon Graphics Octane running an IRIX 6.7 operating system and software package SYBYL 6.9 (Tripos Inc., St. Louis, MO, USA) was used. The structure of charged simazine was minimised to the value of 0.001 kcal mol^{-1} and was screened against a library of 20 polymerisable functional monomers [6]. The Leapfrog algorithm was used to analyse the possible interactions between the template and functional monomers. The program was applied for 30,000 iterations and the results of these were examined and the empirical binding energy score evaluated. This Leapfrog screening produced the list of functional monomers sorted depending on strength of their interactions with template. The monomer with highest the binding energy (MA) and the monomer with the lowest binding energy (HEM) were selected for polymer preparation and further testing.

2.3. Polymer synthesis

The compositions of the imprinted polymers are described in Table 1. The prepared polymer mixture was split into 500-µl aliquots, which were placed in 1.5-ml plastic centrifuge tubes with screw caps containing O-rings (Starlab, Germany). The polymers were prepared by thermo-polymerisation in a silicon oil bath at 80 ºC for 12 hours. The resultant polymer monoliths were removed and used without grinding. HEM-based blank polymer was prepared in the same way as the MIP, but in the absence of template.

2.4. Release studies of simazine in water

In order to monitor the polymer performance, a single polymer monolith was placed in 1 L of distilled water and shaken gently using a KS 250 B shaker (IKA, Germany). The water was exchanged completely every second day.

2.5. Quantification of simazine in solution

The quantification of simazine was performed using Waters HPLC in tandem with a bench-top triple quadrupole mass spectrometer (Micromass Quatro Micro, Waters, UK) equipped with an electrospray probe. The values of the voltages applied to the sampling cone (40 V), capillary (3.2 V), extractor (1 V) and collision cell (20 eV) were optimised by continuous infusion in order to achieve the highest possible sensitivity for simazine. The electrospray probe was maintained at +350 °C with a spray voltage of 450 V for positive ionization mode. The electron multiplier was set at 650 V.

HPLC-MS-MS analyses were carried out in MRM mode, where one daughter fragment (124 m/z) was monitored. The HPLC conditions were: mobile phase A- water, mobile phase B- acetonitrile, both containing 0.1% acetic acid, flow rate- 0.2 ml/min, column- Luna 3 µm, i.d.- 3 mm, length- 50 mm (Phenomenex, UK). The mobile phase protocol was next: 0-10 min- gradient of solution B from 35 % to 100 % and 10-15 min post-run at 35% of solution B. The quantification was performed using MassLynx software and the peak of simazine with t_R= 4.97 min was quantified.

2.6. Kinetic study of simazine release

The kinetic study of simazine release from HEM-based polymers was conducted by placing a single polymer monolith in 1 L of distilled water, which was shaken gently, by a mechanical shaker. In this kinetic study the water was not exchanged and simazine concentration was measured every second day, using the method described above.

2.7. Study of MIP and Blank polymers for re-adsorption and release of simazine

HEM-based MIP and Blank polymers were synthesised as described earlier. The 300-mg monoliths were placed into Soxhlet extractor and washed with methanol for 12 h (approximately 100 washing cycles). The quality of washing was monitored by HPLC-MS-MS using the quantification method described above. Washed polymer monoliths were incubated in the sealed vials containing 10 ml of 0.5 mg mL^{-1} of simazine in methanol or 10 ml of 4 mg mL^{-1} of simazine in DMF, for 3 days at room temperature. Monoliths were transferred into 1 L of water and simazine release was monitored as it was described in sections *2.4* and *2.5.*

3. Results and discussion

3.1. Computer-aided design of MIP for simazine

A molecular model of simazine was designed. The structure was drawn, charged and then minimised to a value of 0.01 kcal mol^{-1}. The molecule of simazine was screened against of the virtual library of functional monomers using Leapfrog algorithm resulting in tables, which rank the monomers with the highest binding score.

Charged MA was found to possess the strongest affinity towards charged simazine (binding energy- 33.89 kcal mol^{-1}). Hydrogen bonds defined the molecular complex between positively charged nitrogen of simazine molecule and negatively charged carboxylic group of methacrylic acid (Fig. 1). HEM was chosen from the Leapfrog table as an example of a functional monomer with relatively weak affinity to simazine (binding energy- 11.93 kcal mol^{-1}). The molecular complex was formed by interactions between the hydroxyl group of neutral HEM and the triazine ring of simazine (Fig. 2). This one-point interaction could be considered as much weaker than the interaction between MA and simazine, which would correlate to Leapfrog's binding score.

The binding energy of the cross-linker (EGDMA), which does not possess any functional groups, was negligible (<-0.04 kcal mol^{-1}). The polymer without functional monomers was made in order to prove that functional monomers were needed in order to control the template release.

According to the results from Leapfrog, it was possible to recommend synthesis and testing of the following polymers (Table 1):

MIP1 (MA-polymer) - molar ratio 1:5 simazine: MA

MIP2 (HEM-polymer) - molar ratio 1:10 simazine: HEM

MIP 3 (EGDMA-polymer) - no monomers, only cross-linker

3.2 Quantification of simazine in solution

The experiment was conducted as described in the Material and Methods. The fragmentation was achieved in positive ionization mode. Multiple reaction monitoring

(MRM) of simazine daughter fragment 124 m/z was performed (Fig. 3). The retention time of simazine was 4.97 min (Fig. 3). The calibration standards of simazine in the range from 1-100 ng mL^{-1} were prepared and injected. The concentration of simazine was calculated accordingly to calibration curves using MassLynx software. Samples which were expected to have higher simazine concentration than range covered by calibration curve (as for EGDMA- polymer), were diluted with water and the quantified concentration was multiplied by the dilution coefficient.

3.3. Release of simazine from MIP monoliths

The results of the simazine release showed a good correlation with molecular modelling. The MA- based polymer demonstrated strong binding towards the template and very slow and steady release of simazine into the water (Fig. 4). The total amount of simazine released over 25 days was calculated as 0.35 mg. Although this polymer could be very good for adsorption of simazine (for example, for solid-phase extraction (SPE) or sensor work) under the specified conditions desorption properties would not be satisfactory for application of the polymer as a carrier of herbicide.

The HEM- based polymer demonstrated lower affinity and released simazine much quicker than MA- based polymer and this correlated with its Leapfrog binding energy (Fig. 4). At the same time, herbicide release was steady and produced a higher simazine concentration in the water, which would be sufficient for practical application. The HEM-based MIP released 10 times more simazine than MA-based MIP. In total, 3.5 mg of simazine were released from 300-mg HEM-based MIP in 25 days. The comparison of simazine desorption from two polymers correlated with the prediction given by computational modelling and demonstrated that using a rational approach to MIP design could help identify a polymer (or a mixture of polymers) with a required rate of desorption.

The EGDMA-based polymer was prepared without any functional monomer and as expected, the release of simazine was the largest and quickest of all the polymers (Fig. 4). During the first week, almost all the simazine was released from EGDMA-based polymer producing a solution with high concentration (at one point up to 2 mg L^{-1}). In total, the EDGMA- based polymer released 5.2 mg of simazine in 13 days. It was possible to draw the conclusion that the presence of a functional

monomer which interacts with the template is required in order to generate a polymer with sufficient affinity to allow the retention of the template and to provide a sustainable release of the herbicide for compensation of natural loses. EGDMA showed negligible interactions with the template and its release was too fast for a potential practical application.

The results demonstrated that by changing the make-up of the polymer we were able to control the rate of release of the template into the solution. The computational approach can be used to design a polymer with a programmable release pattern of the template into environment.

It was observed that during first week the template release from the polymer was much higher than during following days. This tendency could be a consequence of the polymer swelling in water, which caused a release of the template from the surface and some less specific binding sites.

3.4. Kinetics

The HEM-based polymer was selected as the best candidate for practical application; therefore the kinetics of simazine release from HEM-based polymers was studied. A 300-mg polymer monolith was placed in 1 L of distilled water on the mechanical shaker. The water was not exchanged and the simazine concentration was measured every 2-3 days. The resulting curve showed that the saturation point was achieved after 3 weeks and corresponded to simazine solubility in water (Fig. 5). It is worth mentioning that right from the beginning of the period, the quantity of simazine released from HEM- MIP would be sufficient to protect the water from algae growth.

3.5. Evaluation of the simazine re-loading and release

The idea of this experiment was to compare the simazine release from unwashed MIP with simazine release from washed and then re-loaded MIP and blank polymers. HEM-based MIP and blank polymer monoliths were thoroughly washed in a Soxhlet extractor in order to remove the template. The mass-spectrometry measurements showed that no simazine was released from the MIP after Soxhlet extraction, which suggested that simazine was removed completely. In order to load the polymer with simazine, polymer blocks (about 300 mg each) were incubated in 10 mL of 0.5 mg

mL^{-1} of simazine dissolved in methanol. The simazine concentration in the loading solution was determined by its maximum solubility in methanol. The experiment showed that the quantity of simazine released from loaded polymers (0.2 mg from MIP and 0.12 mg from blank polymer) was approximately 20 times lower that released from unwashed MIP. Most likely it was dependant on the amount of simazine which was adsorbed on the polymer. Due to better adsorption of simazine by MIP in comparison with the blank polymer, the amount of released simazine from re-loaded MIP was larger than from blank polymer, but still much lower than from unwashed MIP. Another attempt at loading was made using DMF solution of simazine, which was prepared with a higher simazine concentration of 4 mg mL^{-1} (simazine has greater solubility in DMF as compared with methanol). The polymer was incubated with simazine solution in DMF for 3 days. Then the polymer was transferred into water and simazine release was monitored. It was found that despite the larger quantity of simazine adsorbed from DMF solution than from methanol (Fig. 6), the total quantity of the simazine released into water was still lower than from unwashed MIP. In total only 0.4 mg of simazine was released from MIP and 0.33 mg from the blank polymer. The comparison between MIP and blank polymers showed that the MIP always had higher affinity towards simazine than blank polymer, as a result of imprinting effect. Based on the observations described above it would be possible to draw a conclusion that the most effective way of introducing template into the polymer was to prepare the imprinted polymer in the usual way whereby template is mixed with functional monomers and cross-linker and the polymer mixture is polymerised. The molecular complexation between the template and the rationally selected functional monomers would also allow the introduction of higher concentrations of the template into the polymer than would be possible by physical adsorption.

It was important to find out if components of the polymer mixture other than simazine could be released into the water from unwashed polymer. The list of monitored compounds includes functional monomer (HEM), cross-linker (EGDMA) and solvent (DMF). One monolith of the blank polymer (300 mg) was placed into 1 L of de-ionised water which was changed every 24 h. The aliquot of the solution was filtered and its spectrum was measured and analysed. It was found that no traces of HEM and EGDMA were detected in the first wash, which suggested the polymerisation was effective. As was expected, a large quantity of DMF was present

in the first wash, but this had already reduced significantly (up to 100 times) in the second wash. A short wash of the polymer with water would be sufficient to remove the majority of the solvent molecules from the porous polymeric material. It is also important to highlight that DMF has relatively low toxicity and its presence in minor quantities would not pose a threat to the environment.

Another observation from the experiment was that polymer monoliths were quite fragile and rigid, especially after washing in the Soxhlet extractor. For practical application it might be necessary to incorporate the herbicide-containing polymer particles into polymer composites for better strength and robustness or to grind them into a particle format that could be used within a container. Naturally, the increased surface area resulting would have to be taken into account with respect to the release kinetics.

Conclusions

These results demonstrate for the first time the possibilities of using molecular imprinted polymers for the controlled release of herbicides into water environments such as ponds and aquaria. An optimal rate of herbicide release can be achieved by controlling the amount of polymer and the composition of the MIP. The application of simazine-contained polymer could provide a simple method of day-to-day protection of water without exposing the ecological system to excess simazine and the consequential potential harm.

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Figure legends

Figure 1. A typical view of a pond infested with filamentous and unicellar algae.

Figure 2. Molecular complexes between simazine and MA (left) and simazine and HEM (right). Yellow dotted lines represent hydrogen bonds between charged simazine and functional groups of monomers.

Figure 3. The mass-spectrum of simazine (parent M+1 ion- 202 m/z, monitored daughter ion- 124 m/z); insert: typical chromatogram of simazine during quantification using HPLC-MS-MS.

Figure 4. Simazine release from MA- and HEM-based MIPs and from EGDMA-MIP which was prepared without functional monomers.

Figure 5. Kinetics of simazine release from HEM-based polymer.

Figure 6. Comparison of simazine release from washed and re-loaded MIP and Blank polymers in comparison with simazine release from unwashed MIP.

Table legend

Table 1. Polymer composition.

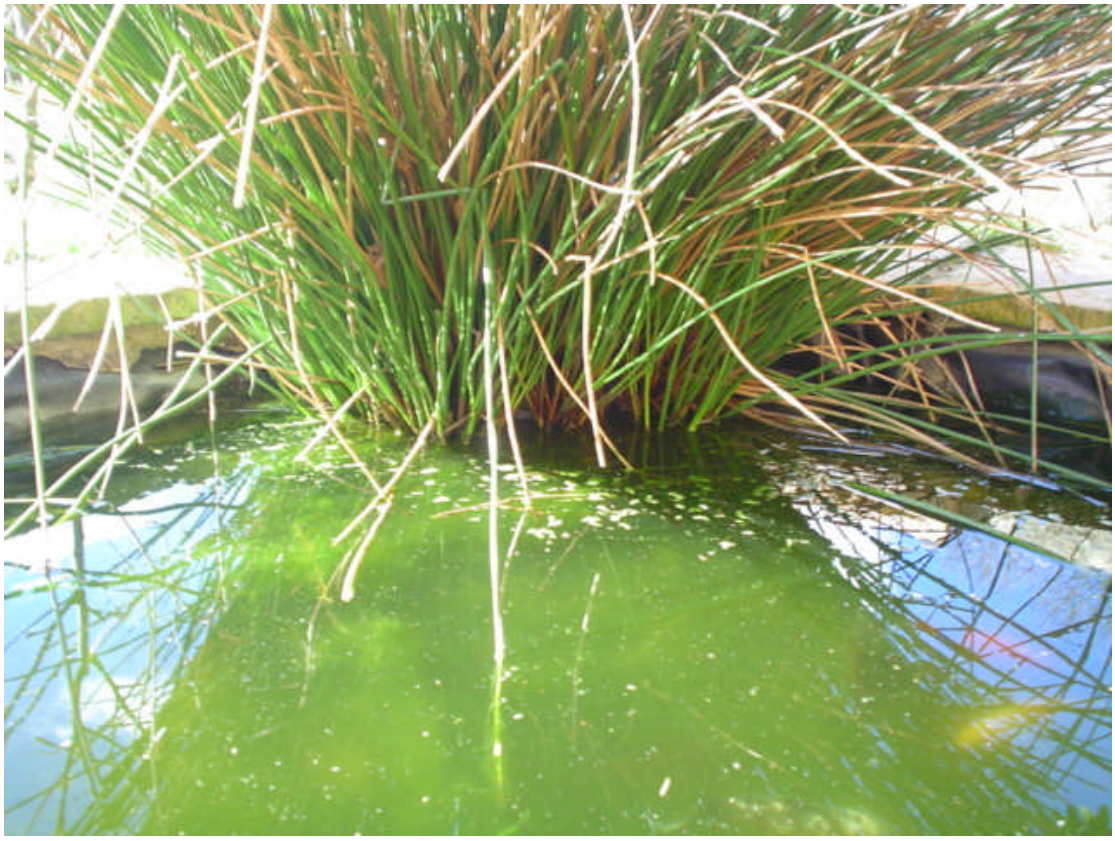
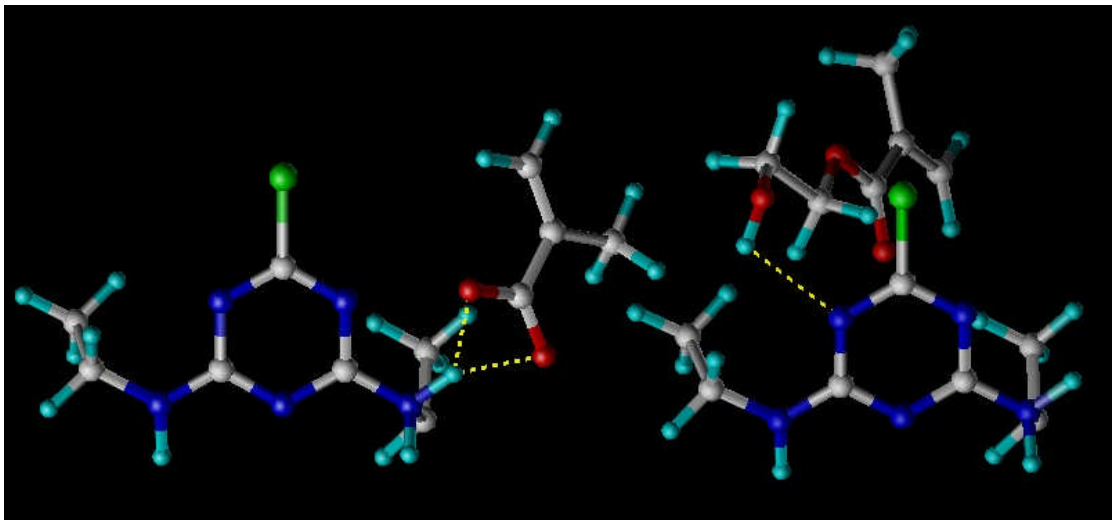


Figure 1. Piletska et al.



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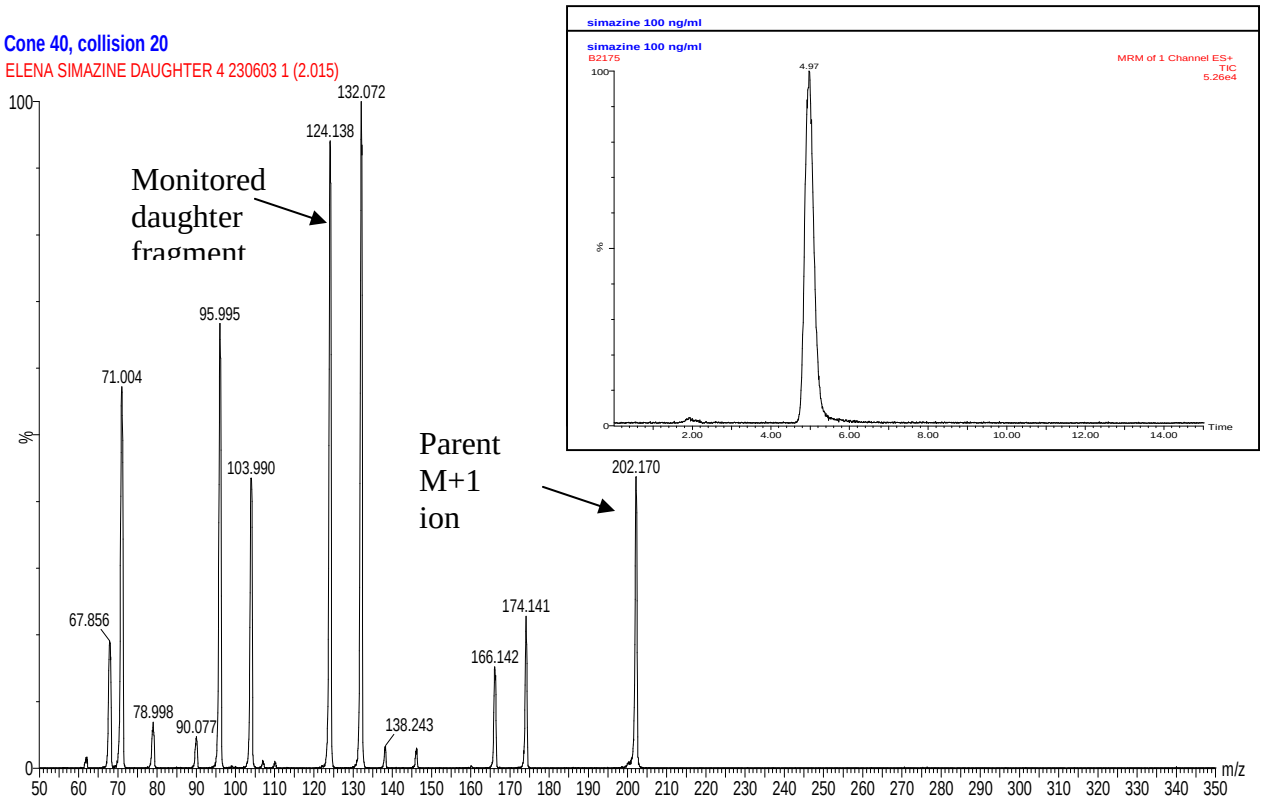

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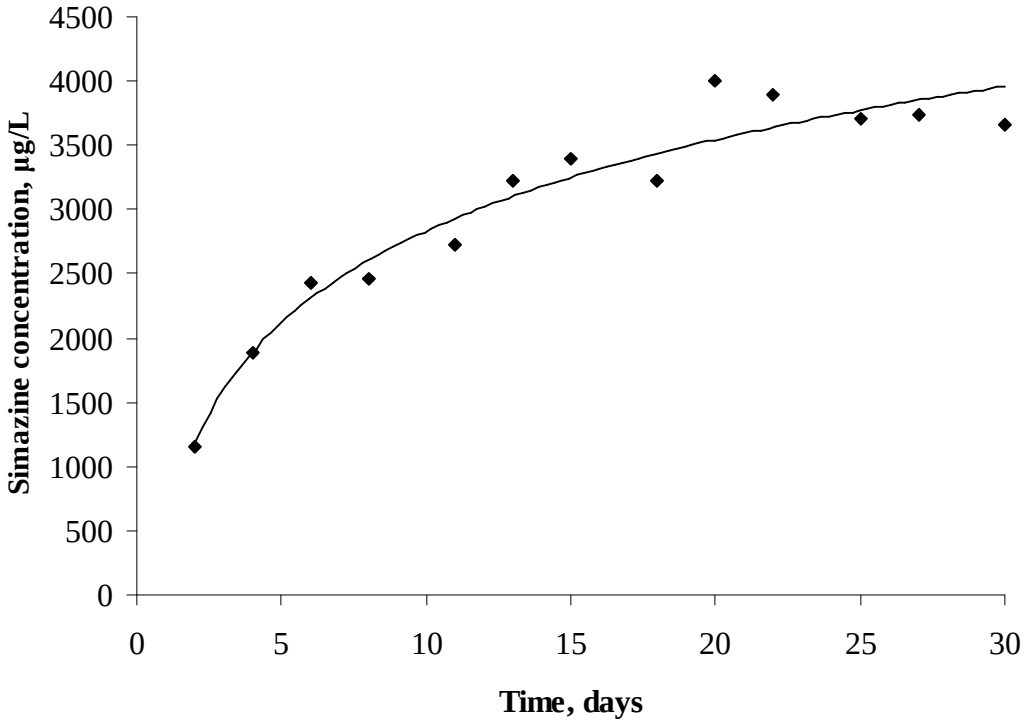

Figure 5. Piletska et al.

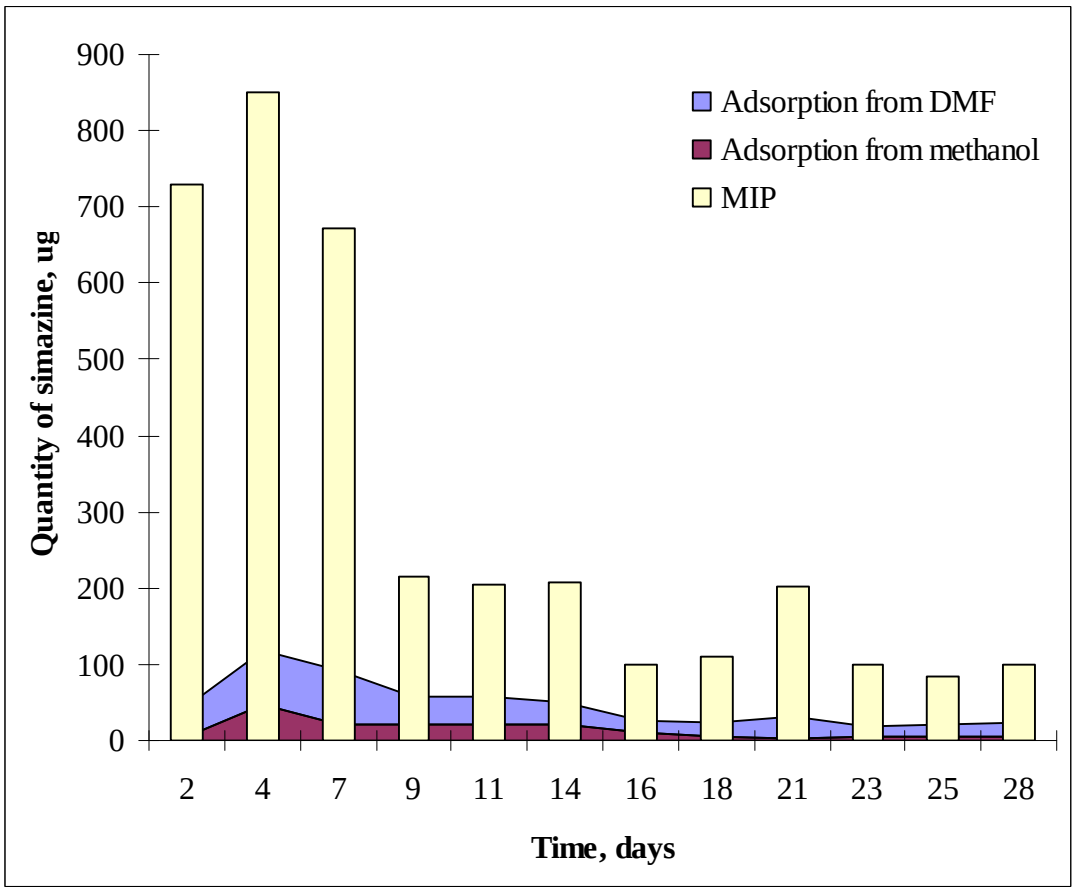

Figure 6. Piletska et al.

Table 1
Polymer composition

Polymer	**MIP1**	**MIP2**	**MIP3**
Simazine, g	0.2	0.2	0.2
Methacrylic acid, g	0.43	-	-
HEM, g	-	1.3	-
EGDMA, g	2.5	6.4	6
DMF, g	3.2	7.5	7.5
Initiator, mg	63	150	150