# Conductimetric sensor for atrazine detection based on molecularly imprinted polymer membranes

**T. A. Sergeyeva,*[a] S. A. Piletsky,[a] A. A. Brovko,[b] E. A. Slinchenko,[b] L. M. Sergeeva,[b] T. L. Panasyuk[a] and A. V. El'skaya[a]**

[a] *Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, 150 Zabolotnogo str., 252143 Kiev, Ukraine. E-mail: sergeeva@biosensor.kiev.ua*
[b] *Institute of Macromolecular Chemistry, National Academy of Sciences of Ukraine, 48 Kharkivske Shosse Str., 252160 Kiev, Ukraine.*

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Atrazine-sensitive conductimetric sensors were designed using molecularly imprinted polymer membranes. Membranes containing artificial recognition sites for atrazine were prepared by copolymerization of methacrylic acid and a cross-linker, tri(ethylene glycol) dimethacrylate, in the presence of atrazine as a template. In order to improve the flexibility and mechanical stability of the membranes, oligourethane acrylate was added to the mixture of monomers. The recognition sites complementary to atrazine were formed in the membranes after extraction of the template molecules with ethanol. Alternatively, reference polymer membranes were prepared with the same monomers but without the template. The responses of the membranes prepared with and without the template were monitored conductimetrically. The membranes prepared in the presence of atrazine showed significantly stronger responses to atrazine than to analogous compounds (triazine, simazine, prometryn). The response time was 6–15 min depending on the membrane thickness. The effect of the membrane composition and the porogen concentration on the magnitude of the conductimetric responses was also investigated. With the sensor designed here, the detection of the atrazine at concentrations down to 5 nmol $dm^{-3}$ was demonstrated. During a 6 month period, the sensitivity of the molecularly imprinted membranes to atrazine was found to remain constant.

The main requirements for detectors of toxic compounds, in particular herbicides, are extremely high sensitivity and specificity. Some standard analytical techniques, such as gas chromatography and mass spectrometry,[1,2] comply with these requirements, thus contributing to progress in the detection of herbicides. Another approach to analyses for herbicides is based on the extraordinary selectivity of antibodies and a number of immunoassays have been developed for the purpose of herbicide determination.[3–5] Despite the excellent sensitivity and selectivity, the uses of such methods are limited because of the relatively long time of analysis and the high equipment cost. To overcome these limitations, alternative techniques have been extensively developed during the last decade. Immunosensors, *i.e.*, portable devices that combine the advantages of antibody-based methods with a low cost and a fast response of miniaturized electronic transducers, seem to represent a very promising approach towards this goal.[6–8]

Unfortunately, the apparent attraction of immunosensors is strongly diminished by three essential drawbacks: (1) a complicated procedure for antibody immobilization; (2) limited stability of any biological material; and (3) poor compatibility of the technology for biological materials and the manufacture of transducers. Also, the preparation of antibodies against low molecular mass compounds requires a hapten to be conjugated with a carrier molecule before immunization. This often results in antibodies with a specificity different from that desired.

These limitations could be overcome by using artificial receptors which mimic the active sites of antibodies. For example, a technique of molecular imprinting enables one to create such active sites in synthetic polymers.[9,10] According to this approach, cross-linked polymers are formed around a template molecule. The template is then removed, thus leaving molecular cavities capable of binding the template molecules back. As has been shown previously, molecularly imprinted polymers (MIPs) can provide high selectivity[11] and sensitivity against haptens.[12] Moreover, the synthesis of MIPs is a straightforward and inexpensive procedure. MIPs demonstrate very good thermal and mechanical stability and can be used in aggressive media.[13] The use of MIPs in sensor applications has been demonstrated in our previous work.[14,15] More recently, the molecular imprinting technique has been successfully applied to the synthesis of polymers that are able to bind atrazine selectively.[12,14,16–18]

This work was focused on the development of a conductimetric sensor based on MIPs specific to atrazine. In particular, we designed and tested simple devices with a free-standing MIP membrane. Their conductimetric responses in the presence of atrazine were analyzed in order to identify optimum technological parameters.

## Experimental

### Materials

Methacrylic acid and tri(ethylene glycol) dimethacrylate (TEDMA) were purchased from Azot (Dnyeprodzerjinsk, Ukraine) and were distilled in vacuum prior to use in order to remove stabilizers. Chloroform was purchased from Sigma (St. Louis, MO, USA), azobisisobutyronitrile (AIBN) from Wako (Osaka, Japan) and atrazine (crystals of 99% purity) from Serva (Heidelberg, Germany). Oligourethane acrylate (OUA) was synthesized as described previously[19,20] and kindly supplied by

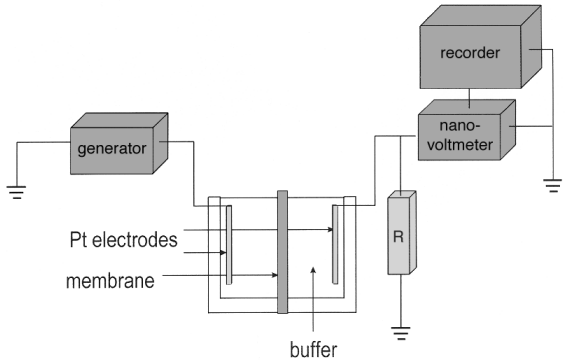



**Fig. 1** Schematic diagram of the experimental set-up for conductimetric measurements.

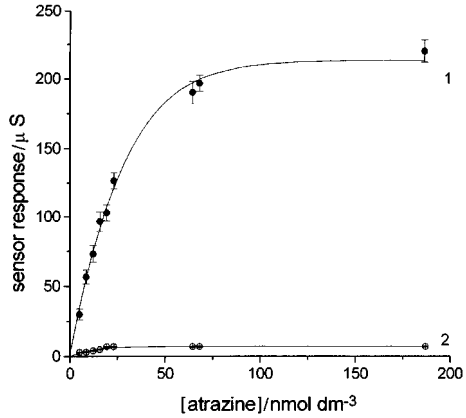
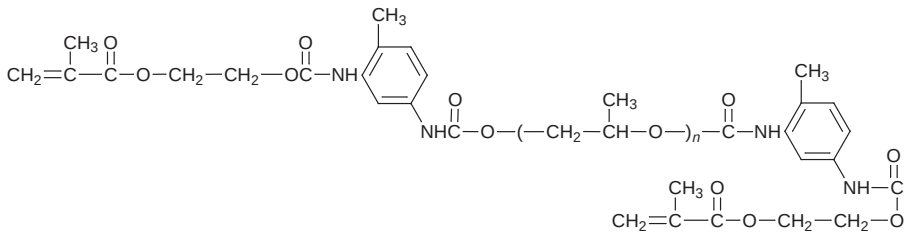



**Fig. 2** Magnitude of conductimetric responses caused by different concentrations of atrazine in (1) MIP membrane and (2) reference membrane. The measurements were carried out in 25 mmol $dm^{-3}$ potassium phosphate buffer (pH 7.5) containing 35 mmol $dm^{-3}$ NaCl. Each point represents the average of three independent measurements.

the Pilot Plant of the Institute of Macromolecular Chemistry (Kiev, Ukraine). Atrazine crystals dissolved in de-ionized water were used as an atrazine stock standard solution for further experiments. Other chemicals were of analytical-reagent grade and were used as received.

### Molecularly imprinted polymer membranes

MIP membranes were prepared using atrazine as a template, methacrylic acid as a functional monomer and TEDMA as a cross-linker. The optimum molar ratio of the functional monomer to the template was estimated from molecular models constructed with Hyper-Chem-3 software and was found to be 5 : 1. In order to obtain thin, flexible and mechanically stable membranes, oligourethane acrylate (molecular mass 2600) was added to the monomer mixture:



where $n$ = 35. A typical preparation of the molecularly imprinted polymer membrane was carried out as follows. A 20 mg amount of atrazine was mixed with 40 mg of methacrylic acid, 289 mg of TEDMA, 51 mg of oligourethane acrylate, 2 mg of AIBN and 30% v/v of the solvent. Then a 60–120 μm gap between two quartz slides was filled with the monomer mixture. To complete polymerization, the slides with the mixture were exposed to UV radiation (365 nm, intensity 20 W $m^{-2}$) for 30 min. After polymerization, atrazine was extracted with ethanol in a Soxhlet apparatus. The 2 h extraction procedure caused no visible changes in the MIP membranes.

The membranes for control experiments were prepared similarly except that the monomer mixture did not contain atrazine.

### Sensor responses

The electrical conductivity of the prepared MIP membranes was monitored in order to evaluate their sensitivity to atrazine. The conductivity measurements were carried out with an electrochemical cell in which two platinum electrodes were separated by the membrane under investigation. Potassium phosphate buffer (25 mmol $dm^{-3}$) (pH 7.5) containing 35 mmol $dm^{-3}$ NaCl was used as a background electrolyte. To avoid electrode polarization, a probing ac signal of small amplitude (60 mV) was used. The probing signal in the frequency range 20 Hz–2 kHz was generated by a waveform generator, G-112/1 (Analytical Equipment, Chernogolovka, Russia). The voltage drop on the resistor connected in series with the cell was recorded with a selective nanovoltmeter, Unipan 233B (Scientific Instruments, Warsaw, Poland) (Fig. 1). The responses were initiated by adding different amounts of the atrazine stock standard solution to the electrochemical cell. The change in the membrane electrical conductivity was then calculated and plotted as a function of atrazine concentration.

Dissociation constants were calculated using Scatchard analysis as described previously.[21] A Scatchard plot was obtained from the calibration curve of the MIP sensor for atrazine using the equation $1/y = 1 + K_{diss}(1/[B])$, where [B] is the concentration of atrazine and $y$ is the ratio of apparent and maximum sensor responses.

## Results and discussion

Covalent interactions between a template and complementary functional monomers have been extensively used in the technology of molecular imprinting.[22,23] According to this approach, a polymerizable derivative of the analyte is synthesized and used as a template. The subsequent extraction of such a polymerizable template requires cleavage of some covalent bonds. Since this substantially reduces the number of potential templates, we focused on another approach that uses non-covalent interactions between a template and functional monomers. In addition to better versatility, this approach allows fast and reversible binding of the template.[24]

In this work, methacrylic acid was chosen as a functional monomer as it is capable of strong electrostatic interactions

(ionic and hydrogen bonds) with atrazine.[25] Most studies with imprinting polymers have been carried out using polymer particles in either a batch or chromatographic mode for the purpose of selective binding. More recently, free-standing membranes of MIPs have been prepared and used for the selective separation of structurally similar compounds,[26–28] and in chemical sensors.[15] However, highly cross-linked polymers tend to yield very brittle and mechanically unstable membranes. In order to improve the mechanical properties of MIP membranes, we used mixtures of the two monomers, methacrylates and oligourethane acrylates, which are considered to yield films with high mechanical stability and reproducibility of the other parameters.[29]

The measured changes in the membrane electrical conductivity as a function of atrazine concentration allowed us to evaluate the recognition properties of the prepared membranes. An increase in atrazine concentration in the solution led to a corresponding increase of the electrical conductivity. At the same time, the reference polymer membranes demonstrated negligible changes of the membrane electrical conductivity in the presence of atrazine (Fig. 2). With the sensor designed here, the detection of the atrazine at concentrations down to 5 nmol $dm^{-3}$ was demonstrated.

It is reasonable to assume that the structure and composition of the prepared MIPs determine their ability to bind atrazine selectively and, thus, the sensor response. Ideally, the polymer should be highly cross-linked so that the atrazine-selective cavities would retain their shapes after removal of the template. At the same time, a certain degree of polymer chain flexibility is important to provide rapid equilibration of the membranes with the template to be recognized. Hence the dependence of the magnitude of the conductimetrically monitored responses was investigated as a function of the amount of cross-linker in the membranes (Fig. 3). It was found that the optimum ratio between TEDMA and oligourethane acrylate in the monomer mixture is 85 : 15 m/m. A further increase in the cross-linker content (up to 90%) decreased the magnitude of the sensor response. The observed conductimetric responses can be attributed to the ability of MIPs to change their conformation upon template binding.[14] Evidence for the template-induced change in the polymer structure has been obtained previously in studies of the non-radiative energy transfer between a fluorescent donor and an acceptor covalently attached to the MIP functional groups.[30] Evidently, the structure of the excessively cross-linked MIP membranes is too rigid and can hardly be affected by interaction with the template. As a result, the sensor responses are low when the degree of cross-linking of the MIP membrane is above optimum.

Good accessibility of the selective cavities formed in a highly cross-linked polymer is extremely important for the membrane to be used as a recognition element of an MIP sensor. This can be achieved when the polymerization is carried out in the presence of an inert solvent. During the polymerization, phase separation takes place and permanent pores are formed in the resulting polymer. The dependence of the membrane recognition properties on the porogen fraction is shown in Fig. 4. As can be seen, the membranes prepared with 25% of the porogen showed very low responses to atrazine. An increase in the solvent fraction up to 30% increased the responses dramatically. However, the membranes obtained in the presence of 40–50% of the porogen were apparently too porous and their electrical conductivity was close to that of the background electrolyte. A more detailed analysis of the polymer morphology and the solvent-induced changes in the membrane structure are under investigation.

Since the best results were obtained with an 85 : 15 m/m TEDMA–oligourethane acrylate mixture containing 30% of the porogen, such a composition was used to prepare the membranes for further experiments.

A series of atrazine analogs (Fig. 5) were used to examine the selectivity of the designed MIP sensor. The responses of the atrazine-imprinted polymer membrane were investigated as a function of atrazine, triazine, simazine and prometryn concentrations (Fig. 6). It was found that these substances in the 1–100 nmol $dm^{-3}$ concentration range generated negligible changes in the membrane electrical conductivity as compared with atrazine.

Using Scatchard analysis, two dissociation constants for atrazine were calculated, $K_d = 1.5 \pm 0.14$ nmol $dm^{-3}$ and $K_d' = 11 \pm 1.4$ nmol $dm^{-3}$. This is in agreement with previous results showing that MIPs, similarly to polyclonal antibodies, contain a heterogeneous population of binding sites with different affinities to the guest molecules.[9] This 'polyclonal' behavior is one of the unavoidable effects of the imprinting procedure when selective cavities are formed owing to the presence of a non-covalently bound template.

The time of the sensor response was found to depend on the membrane thickness. It was 12–15 min for 120 μm membranes; with 60 μm membranes shorter responses (6–10 min) were typically observed. This dependence can be explained by the diffusion-limited kinetics of the sensor responses.

The synthesized membranes demonstrated good stability during prolonged storage. The dry MIP membranes were stored at the room temperature and tested 3–6 months after their

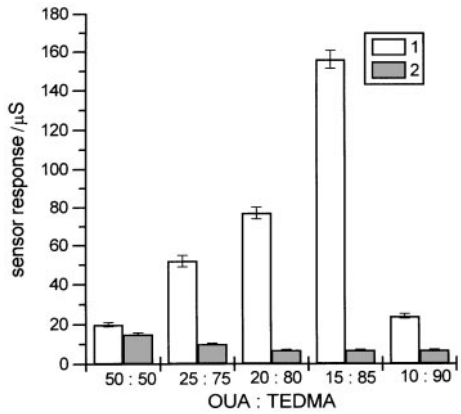



**Fig. 3** Responses to atrazine as a function of the cross-linking agent (TEDMA) concentration: (1) MIP membrane; (2) reference membrane. The measurements were carried out in 25 mmol $dm^{-3}$ potassium phosphate buffer (pH 7.5) containing 35 mmol $dm^{-3}$ NaCl. The responses were initiated by adding 60 nmol $dm^{-3}$ atrazine. Chloroform was used as a porogen. Each value represents the average of three independent measurements.

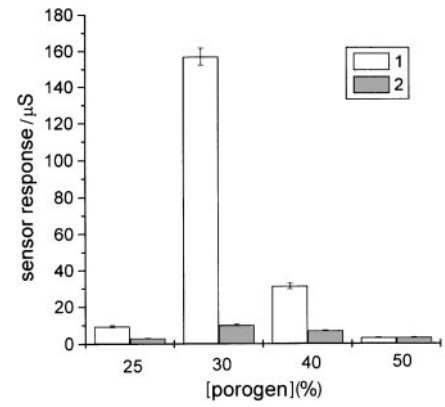



**Fig. 4** Responses to atrazine as a function of the porogen concentration: (1) MIP membrane; (2) reference membrane. Chloroform was used as a porogen. The measurements were carried out in 25 mmol $dm^{-3}$ potassium phosphate buffer (pH 7.5) containing 35 mmol $dm^{-3}$ NaCl. The responses were initiated by adding 60 nmol $dm^{-3}$ atrazine. Each value represents the average of three independent measurements.

preparation, when their sensitivity to atrazine was found to be virtually unchanged.

## Conclusions

A new type of membrane highly sensitive to atrazine was prepared by polymerization of TEDMA–methacrylic acid–oligourethane acrylate mixtures in the presence of atrazine as a template. The resulting molecularly imprinted polymers, similarly to polyclonal antibodies, contain a heterogeneous population of binding sites with different affinities to the guest molecules. The fractions of a cross-linking agent and a solvent added to the monomer mixture have a dramatic effect on the magnitude of the sensor response. Membranes containing 85% of TEDMA and 15% of oligourethane acrylate demonstrated good selectivity and sensitivity and showed rapid attainment of the binding equilibrium. These MIP membranes were successfully used as an active element of a chemical sensor sensitive to atrazine. With the designed MIP-based sensor, the atrazine concentration can be established in 6–10 min. With this sensor, the detection of atrazine at concentrations down to 5 nmol $dm^{-3}$ was demonstrated. High mechanical strength and stability of the studied MIP membranes, together with their inexpensive preparation, provide a good basis for applications of imprinted polymers in sensor technology.

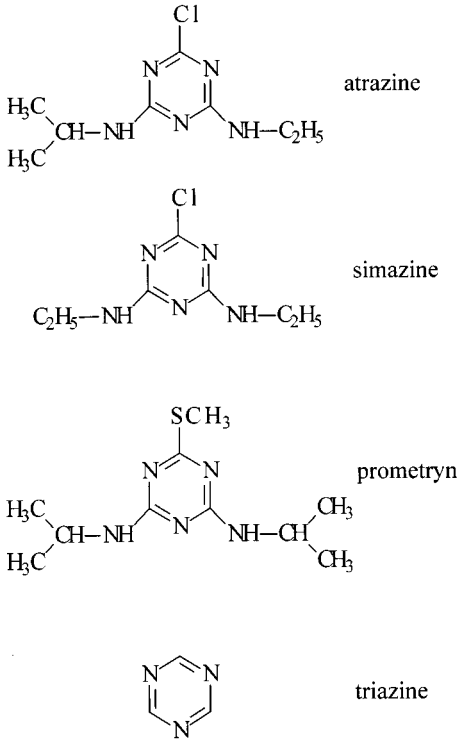



**Fig. 5** Structures of triazine herbicides.

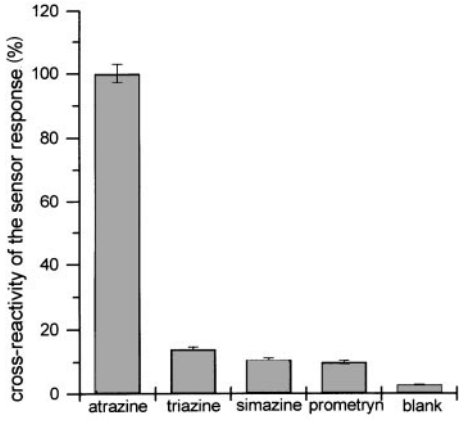



**Fig. 6** Selectivity of the designed atrazine sensor. Atrazine-specific MIP membranes were influenced by atrazine, simazine, triazine and prometryn at a concentration of 70 nmol $dm^{-3}$. Blank, response to 70 nmol $dm^{-3}$ atrazine of a sensor with the reference membrane. The measurements were carried out in 25 mmol $dm^{-3}$ potassium phosphate buffer (pH 7.5) containing 35 mmol $dm^{-3}$ NaCl. Each value represents the average of three independent measurements.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## References


1 A. A. Boydboland, S. Magdic and J. B. Pawliszyn, *Analyst*, 1996, **121**, 929.
2 R. Eisert and K. Levsen, *J. Am. Soc. Mass Spectrom.*, 1995, **6**, 1119.
3 C. D. Watts and B. Hegarty, *Pure Appl. Chem.*, 1995, **67**, 1533.
4 C. Mouvet, S. Broussard, R. Jeannot, C. Maciag, R. Abuknesha and G. Ismail, *Anal. Chim. Acta*, 1995, **311**, 331.
5 M. Franek, V. Kolar and S. A. Eremin, *Anal. Chim. Acta*, 1995, **311**, 349.
6 G. G. Guilbault, B. Hock and R. Schmid, *Biosens. Bioelectron.*, 1992, **7**, 411.
7 F. F. Bier, R. Jockers and R. D. Schmid, *Analyst*, 1994, **119**, 437.
8 A. Brecht, J. Pichler G. Lang and G. Gauglitz, *Anal. Chim. Acta*, 1995, **311**, 289.
9 G. Wulff, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 1812.
10 K. J. Shea, *Trends Polym. Sci.*, 1994, **2**, 166.
11 O. Ramstrom, I. A. Nicholls and K. Mosbach, *Tetrahedron: Asymmetry.*, 1994, **5**, 649.
12 M. Siemann, L. I. Andersson and K. Mosbach, *J. Agric. Food Chem.*, 1996, **44**, 141.
13 D. Kriz and K. Mosbach, *Anal. Chim. Acta*, 1995, **300**, 71.
14 S. A. Piletsky, E. V. Piletskaya, A. V. Elgersma, K. Yano, I. Karube, Yu. P. Parhometz and A. V. El'skaya, *Biosens. Bioelectron.*, 1995, **10**, 959.
15 S. A. Piletsky, T. L. Panasyuk, E. V. Piletskaya, A. V. El'skaya, R. Levi, I. Karube and G. Wulff, *Macromolecules*, 1998, **31**, 2137.
16 J. Matsui, M. Okada, M. Tsuruoka and T. Takeuchi, *Anal. Commun.*, 1997, **34**, 85–87.
17 M. T. Muldoon and L. H. Stanker, *Anal. Chem.*, 1997, **69**, 803.
18 J. Matsui, H. Kubo and T. Takeuchi, *Anal. Sci.*, 1998, **14**, 699.
19 Yu. L. Spirin, Yu. S. Lipatov, V. V. Magdinets, L. M. Sergeeva, Y. Y. Kercha, T. T. Savchenko and L. N. Vilenskaya , *Vysokomol. Soedin., Ser. A*, 1968, **10**, 2116.
20 R. N. Kienle and N. J. Ridgewood, *Germ. Pat.*, 1 085 671, 1961.
21 H. S. Andersson and I. A. Nicholls, *Bioorg. Chem.*, 1997, **25**, 203.
22 G. Wulff, J. Vietmeyer and H.-G. Poll, *Makromol. Chem.*, 1987, **188**, 731.
23 J. Damen and D. C. Neckers, *Tetrahedron Lett.*, 1980, **21**, 1913.
24 O. Ramstrom, L. I. Andersson and K. Mosbach, *J. Org. Chem.*, 1993, **58**, 7562.
25 S. A. Piletsky, E. V. Piletskaya, A. V. El'skaya, R. Levi, K. Yano and I. Karube, *Anal. Lett.*, 1997, **30**, 445.
26 S. A. Piletsky, I. Ya. Dubey, D. M. Fedoryak and V. P. Kukhar, *Biopolym. Kletka,*, 1990, **6**, 55.
27 J. Mathew-Krotz and K. J. Shea, *J. Am. Chem. Soc.*, 1996, **118**, 8154.
28 M. Yoshikava, J. Izumi, T. Ooi, T. Kitao, M. D. Guiver and J. P. Robertson, *Polym. Bull.*, 1998, **40**, 517.
29 L. E. Nielsen, *Mechanical Properties of Polymers and Composites*, Marcel Dekker, New York, 1974.
30 E. Terpetschnig, S. A. Piletsky, E. Pringsheim and O. S. Wolfbeis, in *Applied Fluorescence in Chemistry, Biology and Medicine*, ed. W. Rettig, Springer, Heidelberg, 1998, pp. 277–295.


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