Removal of microcystin-LR from water by polymers based on N-vinylformamide structure
Jan Bialczyk, Andrzej Kochanowski, Urszula Czaja-Prokop and Ewelina Chrapusta

ABSTRACT

The work describes the results of experiments aimed at developing a new method using synthetic polymers to remove microcystins from water. Three hydrophilic polymers were synthesized based on a N-vinylformamide (NVF) structure cross-linked with divinylbenzene (DVB): p(NVF-co-DVB), p(VAm-co-DVB), and, after reaction with glutaraldehyde (GA), p(VAm-co-DVB)GA. The physicochemical characteristics of the resulting polymers were determined by measuring element content, specific surface area, total pore volume and the positive zeta potential of the surface. All the tested polymers showed more than 99.5% adsorption of microcystin-LR (MC-LR) in aqueous solution during 15 min of incubation. Desorption of MC-LR from the complexes formed with polymers depended on the type of polymer and the solvent and ranged from 0 to 22.8%. The yield from application of a field of acoustic waves to methanol solution freed from the MC-LR–p(NVF-co-DVB) complex was 25.1% MC-LR. These results suggest the occurrence of strong bonds in the complex, bonds formed through physical interaction between the negative resultant charge of the MC-LR molecule and the positive charge of the polymer surface. The MC-LR adsorption capacity of the polymer depended on the dose of the toxin: 99.5% at a MC-LR: polymer ratio of 1:1,000 (w/w) and 98.6% at a 1:100 ratio.

Key words | cross-linked polymers, cyanotoxins, microcystin-LR, N-vinylformamide

INTRODUCTION

Eutrophication of surface waters often leads to the periodic occurrence of cyanobacterial blooms. Their bursts of biomass increase present a potential threat to the health and life of humans and animals, as many species of cyanobacteria produce and release toxic secondary metabolites into the environment. The most common toxins in cyanobacteria blooms are hepatotoxic, carcinogenic microcystins (MCs, more than 60 congeners). MCs are synthesized by many species of cyanobacteria belonging to the genera *Microcystis, Planktothrix, Anabaena, Anabaenopsis, Hapalosiphon, Nostoc* and others (Carmichael 1994; Svreck & Smith 2004). Microcystin-LR (MC-LR) is the dominant type of MC worldwide (Haider et al. 2003). It is a cyclic heptapeptide of 994.55 Da molecular weight, composed of seven amino acids: D-alanine (Ala), D-erythro-methylaspartic acid (Masp), D-glutamic acid (Glu), leucine (L), arginine (R), N-methyldehydroalanine (Mdha), and the unusual amino acid (2S, 3S, 8S, 9S)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid (Adda) which is essential for its expression of biological activity (Dawson 1998). MC-LR is an amphipathic molecule with a hydrophobic component in the form of an Adda side chain. The polarity of the MC-LR molecule is due to carboxyl groups of Glu and Masp and amine groups. This configuration gives it high solubility in water. In the range of 3–12 pH, dissociated carboxyl groups impart a negative net charge (−1) to the MC-LR molecule (Lee & Walker 2008). MCs are very persistent compounds resistant to hydrolysis and oxidation, they do not react with acids and bases, and they do not decompose after boiling. They also exhibit resistance to pepsin, trypsin

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© IWA Publishing 2014
doi: 10.2166/ws.2013.192

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and chymotrypsin (Harada & Tsuji 1998). The chemical stability of a microcystin molecule and its high solubility cause many difficulties in removing it from water intended for human consumption. Not all of the methods used in water treatment remove the toxin to the value specified by WHO standards (1 μg/L) (Falconer 1999). MCs undergo biodegradation in the natural environment only after many weeks (Lawton et al. 1995).

In water treatment technology, several methods have been implemented to remove and degrade MCs. Some of them perform poorly. Conventional methods of water purification (floculation, sand filtration, chlorination) eliminate MCs by up to 34% of their initial concentration (Hitzfeld et al. 2000). These and other physical methods (ultra- and nanofiltration) almost completely eliminate the cyanobacterial cells but may simultaneously cause their decay and lysis, releasing the toxin into the water (El Semary 2003). Activated carbon’s adsorption of MCs dissolved in water ranges from 40 to 80% (Bruchet et al. 1998), depending on its origin, structure (Pendleton et al. 2001; Yan et al. 2006), pH and the content of natural organic matter (NOM) in the water (Pendleton et al. 2001). The most effective of the currently used technological methods for degrading MCs in drinking water is ozonation, but at high NOM content large doses of ozone are required, and then the effectiveness of oxidation reaches a maximum of 60% removal of initial MC content, whereas the same ozone dose added to treated water removes 98% of the toxins (Hart & Stott 1993). The performance of other methods in oxidative degradation of MC-LR (Fenton reaction, photocatalysis using TiO₂) is limited by the presence of NOM and/or the cyanobacterial photosynthetic pigment phycocyanin C (Svrcek & Smith 2004). The use of chemical methods (chlorination, ozonation) is associated with the formation of toxic chlorination by-products or semi-oxidation products (Antoniou et al. 2005; Lee & Walker 2008). The use of modified polymers for adsorption and binding of MCs has been the subject of only a few studies, related primarily to the development of ultra- and nanofiltration techniques (Gijsbertsen-Abrahamse et al. 2006). These studies showed that the MC-LR adsorption capacity of a polymer depends on its hydrophobic properties and to some extent on its ability to form hydrogen bonds and its physical surface characteristics (Lee & Walker 2008). Recently, Chen et al. (2011) described a method of forming MC-LR complexes with cyclodextrins by the Adda chain. Such complexation of MC-LR in combination with filtration can significantly increase the efficiency of its removal from water.

The aim of this study was to develop high-performance, easy-to-use and low-cost synthesized polymers to remove MCs from water. We assessed the MC-LR removal efficiency of polymers based on N-vinylformamide (NVF) structure.

### MATERIALS AND METHODS

#### Polymer preparation

NVF was copolymerized with divinylbenzene (DVB) by a reversed emulsion method (water in oil, w/o). The continuous phase was silicon oil (SO), the aqueous phase was NVF and DVB in solution, and the reaction initiator was 2,2’-azobis(2-methylpropionamide)dihydrochloride. The reaction mixture consisting of SO:NVF:DVB (100:9:3, w/w) was placed in a reactor with a mechanical stirrer. The reaction was carried out for 6 h under argon at 65 °C with a stirring rate of 320 rpm. p(NVF-co-DVB) was obtained as a product and washed with toluene to remove the SO and monomers, then dried to constant mass at 40 °C (Figure 1(a)).

![Figure 1](https://iwaponline.com/ws/article-pdf/14/2/230/415468/230.pdf)
hydrolysis of formamide groups in the copolymer was conducted by introducing p(NVF-co-DVB) to 0.1 M NaOH solution in water. The suspension was then mixed for 6 h at 40 °C. The sediment consisting of p(VAm-co-DVB) was drained off, washed with water and dried to constant mass (Figure 1(b)). The p(VAm-co-DVB) polymer was modified by adding 5% water solution of glutaraldehyde (GA). The reaction was carried out for 24 h at room temperature and then the product p(VAm-co-DVB)GA was drained off, washed with water and dried to constant mass at 40 °C (Figure 1(c)).

Element analysis of polymers

The percentages of carbon, hydrogen and nitrogen in each of the obtained polymers were determined with a Euro EA 3000 element analyzer (Euro Vector, Italy).

Determination of polymer texture

To determine the specific surface area ($S_{BET}$) and the pore volume of polymer grains, the BET method and t - plot analysis were used, respectively. The measurements were performed at $-196$ °C using ASAP 2010 adsorption analyzer (Micromeritics, USA). $S_{BET}$ was determined for the relative pressure range ($p/p_0$) from 0.05 to 0.20. Prior to the N$_2$ adsorption the sample was outgassed at 50 °C. The zeta potential ($\zeta$) of the polymers was measured in aqueous solution with a Matec APS acoustic analyzer (USA). A photograph of the polymer was taken with a ProBino DELTA Optical (China) digital microscope at 400x.

MC-LR extraction

MC-LR was extracted from lyophilized cells of *Microcystis aeruginosa* PCC 7813. The conditions for cyanobacteria cultivation and for extraction and purification of MC-LR (purity >98%) were similar to those described by Gajdek *et al.* (2001).

Experimental procedures

MC-LR solution was prepared with Milli-Q water and then 1 mL solution was vortexed with the polymer (100 mg). The experiments were conducted with a horizontal shake (200 rpm) at 25 °C for 5, 15, 30 and 60 min. After incubation the sample was filtered in a glass column (8 mL) with poly-tetrafluoroethylene frit and then eluted with 10 mL Milli-Q water (the first permeate). MC-LR in the permeate was concentrated by solid phase extraction (SPE) with a C$_{18}$ silica cartridge (Baker Bond, Phillipsburg, NJ, USA). The cartridge was conditioned before use and rinsed with 10 mL 100% methanol followed by 10 mL methanol:10% acetic acid (4:1, v/v). Then the aqueous sample was passed through at 1 mL min$^{-1}$ flow rate. After passage through the SPE cartridge the sample was washed with 10 mL portions of 10, 20 and 50% methanol. The eluate, obtained using 10 mL 80% methanol, was evaporated to dryness in a nitrogen stream at room temperature and reconstructed in 1 mL 75% methanol. Samples were stored at $-20$ °C until analysis by high performance liquid chromatography (HPLC). MC-LR adsorbed or bound by polymers was recovered by elution (1 mL/min) with eluents of varying polarity: methanol (ME), acetonitrile (ACN), and 70 mmol phosphate buffer solution of pH 3.2 or pH 10 (the second permeate). Additionally, rinsing tests were performed with the MC-LR–p(NVF-co-DVB) complex using methanol and ultrasound simultaneously. For this we used an ultrasonic washer (Polsonic, Poland) at 40 kHz and 2 × 100 W ultrasound power. The filtrate rinsed from the samples with methanol or acetonitrile was evaporated to dryness in a nitrogen stream; the rest was dissolved in water and concentrated by means of SPE. Samples washed in 70 mmol phosphate buffer solutions were immediately concentrated by SPE, and the subsequent procedure was as described above.

Determination of MC-LR

MC-LR content was determined with an HPLC system (Waters, Milford, MA, USA) consisting of a 600E gradient pump, 717 Plus autosampler, 996 photodiode array detector, Millennium 32 SS software with PDA option, and Jetstream 2 Plus column thermostat. The extracts were separated on a Purospher STAR RP-18 endcapped column (55 mm × 4 mm, 3 μm particles, Merck, Germany), following the method of Meriluoto & Spoof (2005). The gradient mobile phase consisted of acetonitrile/water, both with 0.05% trifluoroacetic acid (v/v), and rose from 25 to 70% acetonitrile in 9 min.
The flow rate was 1 mL/min and the column was maintained at 40°C. Chromatograms were monitored at 200–300 nm. MC-LR was identified by comparing the UV spectra determined for the standard by absorbance at 238 nm.

Calculation of results

The MC-LR adsorption efficiency (AE) of the polymers was calculated from the change in feed concentration in the water (μg/mL) as \( AE = \frac{(c_0 - c_1)}{c_0} \times 100\% \), where \( c_0 \) is the initial concentration of MC-LR and \( c_1 \) is the MC-LR concentration in the first permeate. Desorption efficiency (DE), that is, the efficiency of MC-LR desorption from the MC-LR–polymer complex, was calculated as \( DE = \frac{c_2}{c_0 - c_1} \times 100\% \), where \( c_2 \) is the MC-LR concentration in the second permeate.

Chemicals

All the reagents were of HPLC or analytical grade. Methanol, acetonitrile, trifluoroacetic acid and the MC-LR standard were purchased from Sigma Aldrich (St Louis, MO, USA). Aqueous solutions were prepared with Milli-Q water (Millipore, Bedford, MA, USA). Other chemicals were obtained from LabScan (POCH, Poland).

Statistical analysis

All data are expressed as means ± standard deviation (SD) of five independent experiments.

RESULTS

Physicochemical properties of polymers

To test their adsorption of MC-LR dissolved in water we used three different hydrophilic polymers with NVF cross-linked with DVB. On their surface they contained formamide groups (–NHCHO, p(NVF-co-DVB), Figure 1(a)) after alkaline hydrolysis, amine groups (–NH₂, p(VAm-co-DVB) Figure 1(b)) or, after GA treatment, aldehyde groups (–CHO, p(VAm-co-DVB)GA, Figure 1(c)). The chemical composition and surface properties of the obtained polymers are presented in Table 1.

Changes in \( S_{BET} \) were the result of changes in the percentage content of elements. In the case of the polymer molecule p(NVF-co-DVB) (Table 1), which was examined in detail in most experiments, \( S_{BET} \) was poorly developed, as demonstrated by measurements of polymer \( S_{BET} \) and total pore volume. The p(NVF-co-DVB) polymer averaged 4.4 m²/g \( S_{BET} \), 7.0 \( \times \) 10⁻³ cm³/g total pore volume. For p(VAm-co-DVB)GA we noted a significant increase in \( S_{BET} \) and particle size, resulting from surface digestion after the reaction with GA. All tested polymers also showed positive surface zeta potential (ζ). Figure 2 presents an image of polymer grain with marked dimensions of polymer ball (\( L \approx 30 \mu m \)) and their aggregate (\( L \approx 100 \mu m \)).

<table>
<thead>
<tr>
<th>Polymer</th>
<th>C</th>
<th>H</th>
<th>N</th>
<th>( S_{BET} ) [m²/g]</th>
<th>Total pore volume [cm³/g] ( \times 10^{-3} )</th>
</tr>
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<tr>
<td>p(NVF-co-DVB)</td>
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<td>15.49</td>
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<td>9.59</td>
<td>17.90</td>
<td>4.7</td>
<td>2.8</td>
</tr>
<tr>
<td>p(VAm-co-DVB)GA</td>
<td>58.84</td>
<td>8.62</td>
<td>7.56</td>
<td>24.3</td>
<td>16.3</td>
</tr>
</tbody>
</table>

*\( S_{BET} \), specific surface area of polymer.

Figure 2 | Photograph of polymer grain, p(NVF-co-DVB); L – linear parameter.
MC-LR adsorption

The results for measurement time required to reach MC-LR adsorption equilibrium (10 μg MC-LR/100 mg polymers) are shown in Figure 3. All tested polymers adsorbed more than 99% of the MC-LR from aqueous solution after 15 min incubation.

MC-LR desorption

To determine the stability of MC-LR binding with particular polymers we leached the toxin from the polymer surface with organic solvents differing in the degree of polarity or with phosphate buffers at pH 3.2 or pH 10. Overall, all eluents used were ineffective in releasing MC-LR from the complexes it formed with the polymers, as a maximum 22.8% of the bound toxin was released (Figure 4). The less-polar ACN did not release MC-LR from the complex formed with p(NVF-co-DVB) or from the modified complex p(VAm-co-DVB)GA, and it removed only 0.9% from the MC-LR-p(VAm-co-DVB) complex. Under acidic conditions (phosphate buffer at pH 3.2) 15% of the MC-LR associated with p(VAm-co-DVB)GA was released; 0.3 to 5.0% MC-LR was rinsed from the other complexes (Figure 4). The highest MC-LR recovery (22.8%) was achieved by rinsing MC-LR–p(VAm-co-DVB)GA with alkaline (pH 10) phosphate buffer solution; 12.2% was rinsed from MC-LR–p(NVF-co-DVB) with that solution (Figure 4). Only 1% of the MC-LR was recovered by washing the MC-LR–p(VAm-co-DVB) complex with pH 10 phosphate buffer solution.

In further experiments the p(NVF-co-DVB) polymer was used due to its high binding of MC-LR and low cost of synthesis. The MC-LR–p(NVF-co-DVB) complexes were placed in methanol and treated with ultrasound at 40 kHz. The amount of MC-LR recovered from the complex formed with p(NVF-co-DVB) depended on the duration of ultrasound. After 10 min sonication, 25.1% of the adsorbed MC-LR was released (Figure 5). Extending the duration of ultrasound treatment to 20 min did not have a significant effect on the amount of toxin released from MC-LR–p(NVF-co-DVB).

Adsorption capacity of the polymer

Figure 6 shows the MC-LR adsorption capacity of 100 mg p(NVF-co-DVB) versus the MC-LR dose applied. The
polymer showed strong MC-LR adsorption. At an MC-LR dose of 10 μg or 100 μg, more than 99.5% of it was bound. At the higher MC-LR dose of 1 mg the polymer adsorbed 98.6% (986 mg MC-LR/kg p(NVF-co-DVB)) of the initial toxin content of the aqueous solution.

DISCUSSION

Hydrophilic cross-linked polymers have many and varied applications: in pharmacology as carriers of active substances, in agriculture and environmental protection as preparations for water retention, and in biotechnology as carriers of biomolecules or biocatalysts (Konieczna-Molenda et al. 2009). The positive zeta potential of the polymers determined in the experiments and the resultant negative charge of the MC-LR molecule (Lee & Walker 2013) give clear evidence of the charge compensation of both components. The result of this interaction is stable and strong binding of MC-LR to the polymer surface. This was confirmed by measurements of MC-LR desorption from the complexes formed with the tested polymers: a small amount of MC-LR was recovered by rinsing with solvents of varying polarity (DE 0–22.8% MC-LR, Figure 4), and recovery was similar in the ultrasound treatment (DE 25.1% MC-LR, Figure 5). A comparison with data concerning MC-LR desorption using phosphate buffer at pH 10 (Figure 4), which destroyed hydrogen bonds. Additionally, data concerning MC-LR desorption using phosphate buffer at pH 3.2 (Figure 4) suggest that group –COOH of MC-LR interacts with functional groups on the polymer surface. The physiosorption relies on interaction between the polymer ring and ADDA fragments of the MC-LR molecule. Support for the proposed mechanism comes from results of MC-LR desorption from the polymer complex using organic solvents of various polarity as well as ultrasound (Figure 5).

The use of natural and chemically designed polymers for removal of MCs from water has been the subject of a number of published experimental works. Most of those studies were aimed at developing molecular imprinted polymers (MIPs) as MC detectors for analytical purposes. Chen et al. (2014) discussed MC-LR complexation with cyclodextrins but the efficiency of its removal from water is not reported in that work. Krupadam & Patel (2012) reported that imprinted polymer adsorbents based on itaconic and methacrylic acids adsorbed 85% of MC-LR from an aqueous solution within 45 min. We achieved a maximum 98.6% MC-LR adsorption by p(NVF-co-DVB) (Figure 6) – and also the character and strength of the MC-LR bonds with the polymers. Moreover, the low molecular weight of MC-LR (994.55 Da) and the small size of the molecule when dissolved in water (solvated volume ≈ 2.63 nm³, longest molecular length ≈ 1.9 nm) (Lanaras et al. 1991; Pendleton et al. 2001) might contribute to higher adsorption. The data suggest that an adsorption mechanism similar to that described in the flocculation process (Healy & La Mer 1962) is involved in the interaction of the two components.

The sorption mechanism of MC-LR on tested polymers is of mixed character. It consists of two processes, chemisorption and physiosorption. Chemisorption is conducted by interaction between functional groups coupled on the surface of the polymer (–NH-CHO or –NH2; scheme in Figure 1) and functional groups of MC-LR (–NH-CH-NH2, –COOH) through water molecules and hydrogen bonds. The evidence for this assumption is the results of MC-LR desorption from the MC-LR – polymer complex using phosphate buffer at pH 10 (Figure 4), which destroyed hydrogen bonds. Additionally, data concerning MC-LR desorption using phosphate buffer at pH 3.2 (Figure 4) suggest that group –COOH of MC-LR interacts with functional groups on the polymer surface. The physiosorption relies on interaction between the polymer ring and ADDA fragments of the MC-LR molecule. Support for the proposed mechanism comes from results of MC-LR desorption from the polymer complex using organic solvents of various polarity as well as ultrasound (Figure 5).

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Application of conventional water treatment methods such as sand filtration or coagulation/flocculation caused removal of less than 10% (Chorus & Bartram 2005) or 0–39% (Svrcek & Smith 2004) of microcystin initial concentration, respectively. Polymers based on NVF structure (high capacity and short time of MC-LR adsorption) might be used as an additional adsorbent following mechanical elimination of contaminants by various conventional pre-treatments, such as sand filtration or flocculation. The eventual implementation of proposed polymers in water treatment procedures may play a significant role particularly in small local water stations, where application of advanced methods of microcystin removal is limited. Furthermore, this group of polymers could also be used in domestic jug filtration. Further studies concerning not only practical application but also potential limits of proposed polymers are needed.

CONCLUSIONS

Our results make it plain that synthesized polymers based on NVF structure are potentially an important tool for removal of MCs from drinking water. We demonstrated the high adsorption ability of the tested polymers in treatments to remove MC-LR from aqueous solutions. The fast equilibrium phase of MC-LR adsorption, lasting only a few minutes, is a particularly advantageous feature. This group of polymers based on NVF structure might be used to remove MCs from the water as an add-on to combine with other methods such as sand filtration or flocculation. The next steps in research should aim at explaining the effects of limiting factors on the MC adsorption efficiency of the tested polymers, including water properties such as NOM, mineral content and pH. Another area for study is the polymers’ ability to bind other cyanotoxins.

ACKNOWLEDGEMENTS

This work was partly founded by the Municipal Water and Sewage Enterprise in Kraków.

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First received 28 May 2013; accepted in revised form 8 August 2013. Available online 13 September 2013.