Determination of Microcystins in River Waters Using Microsensor Arrays on Disk

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Received May 14, 2010. Revised manuscript received September 16, 2010. Accepted October 12, 2010.

The development of simple, accurate, and rapid multisample analytical methodologies to find out critical targets in waters is highly demanded. Optical microsensor arrays to determine microcystins in river waters are developed on the polycarbonate side of compact discs. The working principle of the sensors relied on an indirect competitive microimmunoassay, where free microcystin LR (MC-LR) competes with immobilized conjugate for specific monoclonal antibody. The results of the immunoreaction are detected with a DVD drive, showing the readouts in minutes. The method reached a sensitivity (IC_{50}) for MC-LR of 1.04 μg/L and a linear response in the range 0.12−2.00 μg/L, allowing its determination below the upper limit proposed by the World Health Organization in drinking water. The developed analytical approach shows simplicity, good sensitivity, high throughput capability, and rapidity (37 min) in field use. The optimized assay showed also high congener reactivity to MC-LY (144%), MC-LA (125%), MC-LF (119%), MC-LW (102%), MC-YR (83%), and nodularin (94%). Furthermore, the suitability of the disk biosensor to quantify MC-LR was successfully evaluated analyzing river water samples, obtaining excellent recoveries (78−113%). Precoated discs are stable for at least seven weeks without loosing their analytical performances. Also, the portability of the analytical system permits on-site analysis and quantification, saving time and other resources. To our knowledge, this is the only work where a portable, easy-to-use, array based system has been developed for on-site microcystin quantification and applied to simultaneously analyze 42 samples plus the calibration curve, reaching microgram per liter sensitivity.

Introduction

The interest of developing sensor systems for practical use in different scenarios has noticeably increased in recent years. Certainly, biosensors are currently used in clinical diagnostics, genomics, and proteomics, as well as in environmental monitoring. In the last mentioned field, research has been focused toward the combination of low-cost, disposable platforms and high-throughput sensitive microassays with robust and portable detection systems to operate out of laboratory facilities (*1*).

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These requirements are not always easy to meet since in general the detection devices suffer from high-cost, large size, and lack of portability. At present, many integrated optical systems are being explored in which waveguides and optoelectronic elements are incorporated, improving sensitivity while reducing costs. In this perspective, electronic gadgets for consumer applications are attracting significant attention for optical sensing. In particular, optoelectronic components of standard disk drives have cost advantage over conventional optical detectors. In line with this, several applications in life sciences based on optical disk technologies have been reported, either with additional photodetectors for transmission measurements (*2*−*5*) or as separate detection units (*6*−*9*).

The use of conventional discs as sensing platforms in combination with the potential of biochemical methods in microarray format using disk drive has been emerged for the development of interesting and practical analytical approaches (*10*−*13*).

In this work, microsensor arrays on commercial DVDs as a low-cost platform with a portable and hand-held detector to quantitatively determine microcystin LR (MC-LR) in river waters is addressed.

The determination of these biotoxins is an interesting matter to deal with since most of the world's population relies on natural waters as its primary source for drinking water (*14*). Fast quantitative determination of these compounds is essential to ensure water quality, and therefore, human health (*15*). To this end, the World Health Organization (WHO) have proposed a provisional upper limit for the most toxic microcystin (MC-LR) of 1.0 μg/L in drinking water (*16*).

On a regular basis, microcystins are precisely identified using high performance liquid chromatography coupled to mass spectrometry (LC/MS), using triple quadrupole, ion trap and matrix-assisted laser desorption/ionization time-of-flight (MALDI-ToF) detectors (*17*). These methodologies require expensive equipments, long procedures, and analysis times. Also, the large number of more than 80 congeners represents a considerable challenge for the analysis of MCs because standards are not always commercially available and the concentrations of the individual components may be below the limits of detection (*18*). Unlike this methodology, immuno-based tests are rapid, easier, and have been addressed as the most promising methods for microcystins (*19*). Nowadays, a variety of immunological methods to measure microcystin concentrations are available. For instance, the commercially existing ELISA kits can accommodate several samples, plus standards and blanks, reaching enough sensitivity to determine microcystins at μg/L level.

In the last year, several screening biosensing methods have been also reported for microcystins analysis (*19*−*22*). These approaches differ in sensitivity, cost, and technical complexity. The assays test one sample at a time, requiring sensor surface regeneration between each cycle, increasing the analysis time. Besides, the sample volume is hundreds of microliters, becoming larger the volume of immunoreagents needed. Also, biosensing methods or lab-on-a-chip techniques for simultaneous analysis of several samples have not been proposed.

To circumvent these issues, we reported multiplexed microimmunoassays on a digital versatile disk (DVD) that allowed for using the DVD technology to detect and quantify different analytes simultaneously (*12*). In that study, the maximum number of samples analyzed per disk was eight and performed in segmented areas. In the present work, the

10.1021/es101653r

Published on Web 11/03/2010

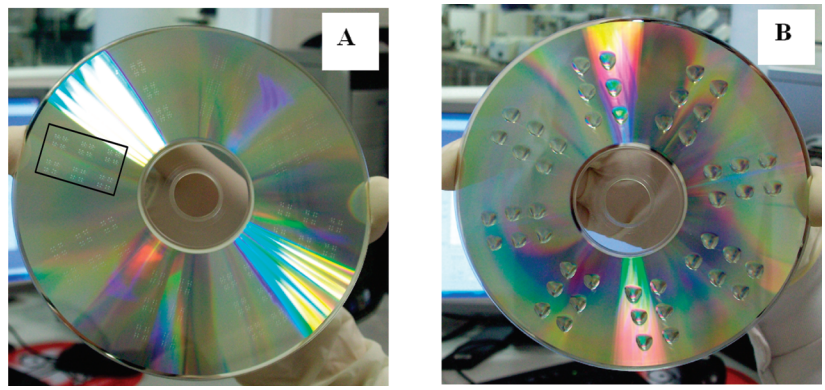

FIGURE 1. Pictures of the microsensor array on disk layout. (A) The disk holds eight sections of six arrays of four blocks (2 × 2 spots) each. One of these sections is framed for better visualization. In total 48 arrays were printed on the disk surface. From the inner to the outer of the disk, the left-down, right-down, left-up, and right-up blocks correspond to biotin, microcystin systems, MIgG and RIgG as positive controls, respectively. (B) Image of a disk after 8 min in vertical position. A volume of 10 μL of antibodies cocktail solution in PBST was dispensed. The hydrophobic nature of the polycarbonate side of the disk, the appropriate array distance and the low volume used prevents cross-contamination of samples.

high-throughput capability of the system is improved, accommodating, as proof of concept, 48 samples, including an external and internal calibration and positive control spots. Besides, the portability of the analytical system allows on-site analysis and quantification, saving time and other resources.

Experimental Section

Chemicals. Printing buffer, 0.1 M sodium carbonate buffer, pH 9.6 containing 1% glycerol (v/v), analysis buffer, 10 mM sodium phosphate buffer 0.15 M NaCl, 0.005% Tween 20, pH 7.4, and washing solutions were filtered through a 0.22 μm pore size disk before use. The coating conjugate (BSA-MC) and the monoclonal antibody (mAb-MC) were kindly provided by Abraxis kits (Warminster, PA). The standards MC-LR, MC-LA, MC-LF, MC-LW, MC-LY, MC-YR, and nodularin were also from Abraxis kits. Rabbit polyclonal antibiotin antibody (PAb-BT) was from Abcam (Cambridge, UK). Biotin, biotin N-succinimidyl ester (used to prepare OVA-biotin conjugate; OVA-BT), gold labeled goat antirabbit and anti-mouse immunoglobulins (GAR-Au and GAM-Au, respectively), and silver enhancer solutions (A and B) were from Sigma-Aldrich (Madrid, Spain). Note: The microcystins standards and silver enhancer solutions and solvents must be handled following their material safety data sheets.

Microarray Printing. Bulk DVD-r discs were purchased from MPO Ibérica (Madrid, Spain). The discs were first conditioned by gentle ethanol washing, water rinsing, and dried by centrifugation. The coating reagents, diluted in printing buffer, were dispensed in a 384-well plate (25 μL/well) and transferred to the disk (25 nL) with a noncontact printing device (AD 1500 BioDot, Inc., Irvine, CA). The disk layout (Figure 1A) consisted in eight zones (45° separation) with six microarrays (at a distance of 9 mm, corresponding to the space between nozzles in standard multichannel pipettes) of four subarrays each, arranged in blocks of 2 × 2 spots. A total of 48 microarrays were printed on the polycarbonate surface under controlled temperature and relative humidity (25 °C and 80%, respectively). Within each microarray, the blocks correspond to internal standard (OVA-BT) and microcystins systems (BSA-MC), and two positive controls, respectively. In this configuration, spots are 350 μm in diameter with a track pitch (center to center distance) of 0.9 mm.

Microimmunoassay Protocol. The working principle of the sensor arrays on disk was based on an indirect competitive microimmunoassay format. Competition occurs between free microcystin-LR (sample) and immobilized conjugate for specific monoclonal antibody against MC-LR (mAb-MC). To this end, BSA-MC at 30 mg/L, OVA-BT at 0.2 mg/L for internal calibration, and nonimmunized mouse (MIgG) and rabbit sera (RIgG) at 1/1000 and 1/4000 dilution, respectively, as positive controls were arrayed on the polycarbonate surface of DVD-r discs. After 16 h at 4 °C, discs were thoroughly washed with PBS-T, rinsed with deionized water, dried by hand shaking, and stored at 4 °C until use.

For MC-LR assay, different sample volumes (5, 10, 15, and 20 μL) were tested. After optimization, 10 μL of antibodies cocktail solution (1/5000 dilution for mAb-MC and 1/1000 dilution for PAb-BT) in PBS-T, with and without analyte was dispensed onto the array. After 8 min incubation at room temperature, the disk was washed with deionized water. Next, 500 μL of gold-labeled secondary cocktail antibody solution (1/50 for GAM-Au and 1/250 for GAR-Au, in PBS-T) was dispensed onto the disk, and it was evenly distributed using a dummy plastic surface. After 15 min at room temperature, the cover surface was removed, and the disk washed and dried as before. The immunoreaction was developed by dispensing 1.0 mL of silver enhancer solution onto the disk and distributed as before. Finally, the reaction was stopped after 8 min by washing the disk with water. As is shown in Table 1 the total assay takes 37 min.

The selectivity of the microsensors was determined against several microcystins congeners and the cross-reactivity was calculated by the following equation: $[IC_{50}\ (\text{MC-LR})/IC_{50}\ (\text{congener})] \times 100$.

Stability of the Microsensors. The activity of precoated disk and that of the immunocomplex were tracked during seven weeks and seven days, respectively. For that, the signal intensity obtained at time zero, used as reference, was compared with that obtained at different times, expressing the result in percentage.

Disc Imaging and Data Analysis. The DVD drive used in this study was from LG Electronics Inc. (Englewood Cliffs, NJ), which was controlled by custom software (Diskpick) running on a personal computer, and connected to it through a USB2.0 universal serial bus interface as previously described (*12*). The challenges in the implementation of this detection system for sensing purposes were overcome with our disk drive approach (*9*). In the sensor system, there are no optical modifications of the drive; thus, the optical disk reader performs its original function of scanning the whole surface of the optical media. Also, it provides an analog signal for quantitative analysis when microarrays are deposited onto the read side of a compact disk.

Briefly, during the DVD reading, the laser (λ 650 nm) hits the immunoreaction product that modifies the reflection properties of the DVD surface, attenuating the laser beam intensity that reaches the photodiode of the pickup. The analog signals are directly acquired from the photodiode of the disk drive, being related to optical density of the reaction product which is inversely proportional to analyte concentration.

To scan the surface of the DVD completely (5 min at 16× speed), the software simulates the writing process of a 3.8 GB size file. During the disk scanning, only signals coming from selected areas are processed for digitization, stored in the computer (5 MB size file), and deconvoluted into an image. Diskpick software was written in Visual C++ to control the optical disk drive, the data acquisition board (sampling rate, detector gain, spatial resolution, and scanning speed) and identifies spots with a signal-to-noise ratio ($S/N \geq 2$. To calculate the mean signal intensity of the spot, the program averages data points from a circular area of 50 μm in diameter. Moreover, this software allows for exporting the results. Inhibition responses were mathematically analyzed by fitting experimental results to a linear regression curve.

Analysis of River Water Samples. For analysis of river waters, the samples were conditioned by mixing nine parts by volume of river water with one part by volume of 10-fold concentrated PBST. To this mixture, the appropriate antibody cocktail solution was added. Then, the immunoassay was performed as described above.

Jucar and Turia River water samples ($n = 12$) obtained from the Valencia metropolitan area water supply corporation (Aguas de Valencia, S.A.) were analyzed with the microsensor arrays on disk approach. The chemical composition of Jucar and Turia river waters is shown in Table 2. All water samples were microcystins free (confirmed by LC/MS reference method (*23*) and ELISA test kit from Strategic Diagnostics, Inc., Newark, DE). After filtration through a 0.22-μm pore size disk, samples were stored at 4 °C prior to use. Before analysis, samples were spiked with MC-LR at different levels, within the linearity range and determined directly without previous extraction. Each sample was analyzed in duplicate in the same disk (8 replicate spots measured per sample). Finally, the disk was read by the DVD drive giving quantitative results.

Results and Discussion

Microsensor Arrays Performance. The majority of reported on-site screening methods for microcystins analyze one sample at one time. In our work, we design sensor arrays on discs, including as proof of concept 48 arrays for the simultaneous analysis of 42 samples, including also calibration and control arrays. In the presented configuration, each array contains four blocks (subarrays) of 2 × 2 spots. One of them is dedicated to the analysis of microcystins, whereas the rest operate as positive controls of the immunoreaction and detection steps.

As far as the microcystin system optimization is concerned, different concentrations of coating conjugate BSA-MC, ranging from 0.05 to 50 mg/L, were tested against serial dilutions of mAb-MC (1/500 to 1/50 000). Optimal coating conjugate concentrations and antibody dilutions were selected on the basis of signal intensity and signal-to-noise ratio ($S/N > 35$) by check-board titration in a competitive format. The optimum concentration was found to be 30 mg/L for the coating conjugate and 1/5000 dilution for the monoclonal antibody.

The role of the subarray for biotin system was 2-fold. First, it works as an internal calibration system as previously described (*12*), and second it performs as positive control of the immunoreaction step. To this end, a cocktail solution containing mAb-MC and PAb-BT was prepared. Thus, no readings at the control indicate that the assay was not successfully completed and serves as an alarm quality control test.

TABLE 1. Assay Steps and Time Taken for a Competitive Microcystin Array on Disc

assay steps	time (min)
sample-antibody reaction	8
washing	0.25
secondary antibodies reaction	15
washing	0.25
signal amplification	8
washing	0.25
disc reading	5
total assay time	37

Alternatively, MIgG and RIgG work as positive controls of the detection step, due to the ability of gold-labeled secondary antibody cocktail solution, used as tracer, to recognize rabbit and mouse sera. For these experiments, different concentrations of OVA-BT ranging from 0.01 to 1.00 mg/L and different MIgG and RIgG dilutions (1/1000 to 1/10 000) were tested. In terms of signal intensity and reproducibility, the optimum concentration of reagents was found to be 0.2 mg/L for OVA-BT, and 1/1000 and 1/4000 dilution for MIgG and RIgG, respectively.

To test the suitability of the controls, the sensor was run in the presence of different MC-LR concentrations. The signal intensities obtained were similar to that reached in the absence of microcystins. The intradisc relative standard deviation (RSD) varied from 1.8% to 2.6% whereas the interdisc RSD ranged from 3.3% to 5.2%, indicating that MIgG and RIgG appropriately perform the role of positive controls. On the basis of these figures, the positive controls can be used to correct assay variability. For instance, disk area variations that might cause a lower/higher-than-normal reading at the controls would have the same effect on the microcystin array block.

The sample volume was also another parameter studied. For these experiments, four volumes (5, 10, 15, and 20 μL) were assessed in a noncompetitive immunoassay. The selection criteria were signal intensity and reproducibility, measured the former as relative standard deviation. For the blanks (absence of analyte), the signal was significantly similar for all the tested volumes, varying from 14 580 to 16 590 (arbitrary units) for 5 and 20 μL, respectively. The test performed to determine significant differences was a single-factor ANOVA analysis, obtaining a level of signification (α) of 0.07. In terms of signal reproducibility, the best results were obtained with 10, 15, and 20 μL solutions (RSD below 10%). The explanation of this finding arises from the spot size. In the disk layout, the array containing the four blocks was 4.5 × 4.5 mm in size, and spots of 10 μL of PBS-T solution had a diameter of 4.8 mm, whereas spots of 5, 15, and 20 μL of the same solution were of 3.4, 5.6, and 6.4 mm in diameter, respectively. After 8 min, the spots were spread about 1.0 mm in diameter, 15 and 20 μL of PBS-T solutions occupying circular areas very close each other. For all of these reasons, 10 μL sample solution was selected for further analysis in the present disk layout (see Figure 1B), covering the whole array surface and preventing cross-contamination between flanking samples.

Under the optimal conditions (30 mg/L and 1/5000 dilution of coating conjugate and monoclonal antibody, respectively) and working times (see Table 1), the assay reached a sensitivity, calculated as the midpoint of the inhibition curve (IC_{50}), of 1.04 μg/L with a linear test ranging from 0.12−2.00 μg/L using six standards, as is shown in Figure 2A. A good linear correlation was fitted with r^2 value 0.991.

TABLE 2. Chemical Composition of River Water Samples[a]

parameter	Jucar	Turia
pH	7.76	7.85
dry residue (mg/L)	656	639
conductivity at 25 °C (μS/cm)	1650	1765
organic matter (mg O_2/L)	2.5	2.8
bicarbonate (mg/L)	188.4	186.3
chloride (mg/L)	101.7	115.2
sodium (mg/L)	108.2	116.3
calcium (mg/L)	79.5	98.5
magnesium (mg/L)	44.0	37.9
ammonium (mg/L)	0.12	0.11
nickel (μg/L)	19.5	20.3
zinc (μg/L)	13.2	16.0
iron (μg/L)	56.2	58.6

[a] The figures are mean values.

These figures allow the determination of MC-LR below the upper limit established by the WHO. Representative optical density images of a competitive microsensor array are shown in Figure 2B. As it can be observed, the signal intensity decreases as MC-LR concentration increases, following the principle of a competitive immunoassay. Also, the positive controls confirm the proper function of the microsensor arrays.

Specificity of the biosensor was evaluated determining the cross-reactivity of related compounds. To this end, competitive calibration curves in PBST for other microcystins were developed. Cross-reactivity (CR) was calculated as the percent ratio at the midpoint of the assay between MC-LR and the cross-reacting compounds. The most interfering microcystin was MC-LY (IC_{50} 0.72 μg/L) with CR 144%, whereas cross-reactivity for MC-LA, MC-LF, MC-LW, MC-YR, and nodularin was 125%, 119%, 102%, 83%, and 94%, respectively. These results indicate that the sensor system might accomplish the determination of an overall content of microcystins, displaying both high-congener reactivity and high sensitivity.

The peptidic nature of microcystins (MW: 995−1068 Da) allows for their direct immobilization on the polycarbonate disk surface by physical adsorption, maintaining the bio-disponibility of the peptide. This immobilization strategy circumvents the need for coating conjugate preparation. For these experiments, MC-LR concentration ranging from 0.01 mg/L to 1.0 mg/L was immobilized directly onto the disk. The assay was run in the same manner as described before for the coating conjugate based sensor. The experimental results fitted well ($r^2 = 0.997$) to a linear regression curve with equation $y = -5761x + 5090$, x being the log of microcystin concentration (see Figure 2A). The working interval of the sensor test ranged from 0.25 μg/L to 1.50 μg/L, using four standards, reaching a sensitivity (IC_{50}) of 0.92 μg/L. Though the signal intensity of the assay was lower (65% in absence of analyte) than that based on the immobilization of the protein conjugate, the slightly lower IC_{50} makes this assay capable to determine the most toxic microcystin in the μg/L level.

Stability of the Microsensor Arrays on Disk. Though the disk can be used as a disposable platform, it is also very interesting to follow the stability of the precoated disk and that of the immunocomplex from a practical point of view. To this end, the activity of the sensor for the determination of microcystins was studied.

The activity of the precoated disk to be further recognized by mAb-MC was tracked during seven weeks. For these experiments, each section (see Figure 1A) was analyzed every week using freshly prepared mAb-MC. As is shown in Figure 3, the activity of precoated disk was maintained until seven weeks. Thus, taken the signal intensity obtained in the assay

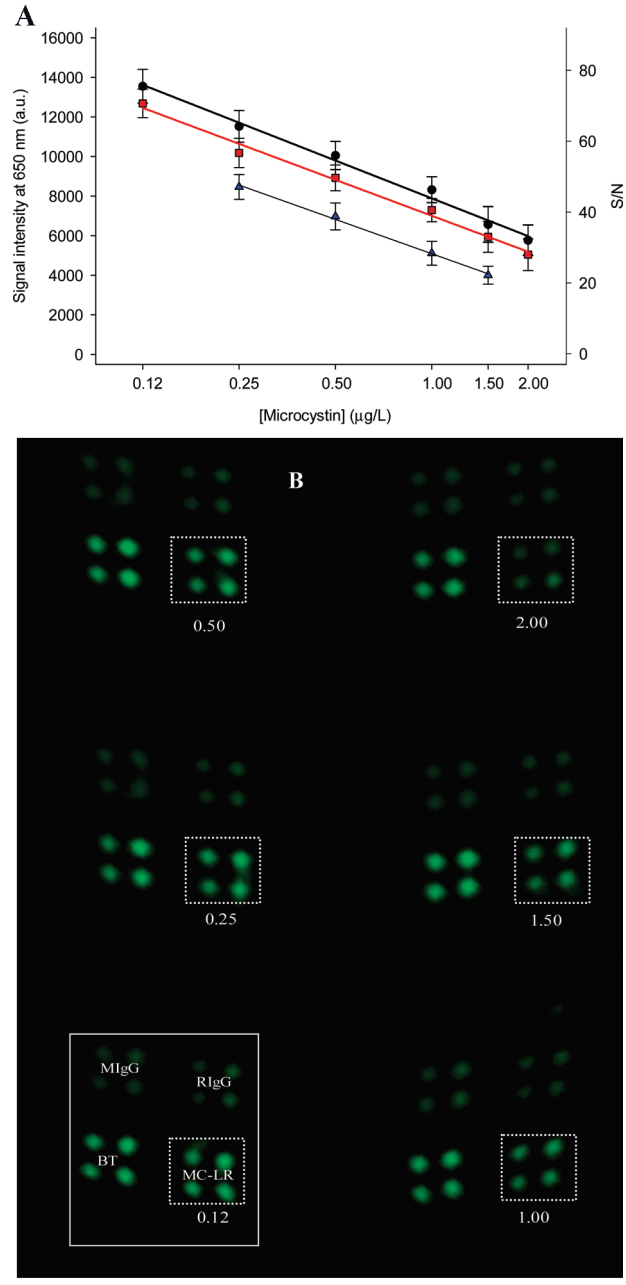

FIGURE 2. Competitive microsensor arrays for microcystin LR.(A) Standard calibration curves of MC-LR obtained in PBST (●), mixture of twelve river water from the Jucar and Turia rivers (□) and in PBST coating the disk directly with MC-LR (1.0 mg/L) (▲). The curves are the mean of twelve plots obtained in different days. *S/N*: Signal-to-noise ratio. (B) Optical density images of a competitive microsensor immunoarray performed on DVD-r.The figures below the framed squares are the microcystin standard concentrations in μg/L. The left-down, right-down, left-up, and right-up blocks correspond to biotin (BT), microcystin systems (MC-LR), nonimmunized mouse (MIgG) and rabbit (RIgG) sera as positive controls, respectively.

performed on week 0 (100%) as reference, the intensity profile for the period tested indicated that coating probe immobilized onto the disk was active for at least 50 days without loosing sensor performances. During the experimental period, the discs were stored inside a slim box at 4 °C in the refrigerator. The stability and robustness of the precoated disk is promising, which is also indicative of its reliability and cost-effectiveness in terms of MC-LR measurement. But, from a practical point of view more work should be performed

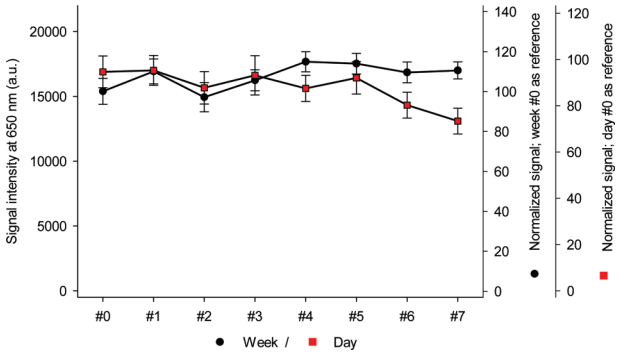

FIGURE 3. Stability profile of the precoated disk (●) and of the immunocomplex formed with mAb-MC (□).

to increase the stability of the precoated discs, maintaining the analytical performances.

Also, the activity of the immunocomplex was studied for a period of seven days. For that, each section was analyzed every day. First, the immunorecognition reaction was carried out in all sections (day 0). Then, after immunoreaction and washing step, the discs were stored at 4 °C until analysis. To this end, each section was detected using the secondary cocktail solution as tracer and the results displayed (silver amplification method) in different days (1−7). As is shown in Figure 3, the immunocomplex immobilized on the disk retained 90% of its original response during at least 5 days, observing a decrease after the seventh day (73% of the original response was maintained). These results show that the shelf life of the immunocomplex is limited, so in remote field sites the assay should be completed within one week to get reproducible results.

Water Sample Analysis. The effect of sample matrix on sensor system performance was studied. To this end, standard curves were prepared in spiked river water samples ($n = 12$). The water samples were from Jucar (W 1−3; W 7−9) and Turia rivers (W 4−6; W 10−12). The mean curve was compared to that obtained in PBST, to elucidate whether the linear calibration in PBST could be used in the analysis of real samples. As is shown in Figure 2, the effect of water matrix was almost negligible, according to the intercept and slope values obtained from the linear regression equation (water, $y = -6036x + 7007$; PBST, $y = -6345x + 7888$). Also, the calibration curve in river water reveals similar sensitivity (IC_{50} 0.96 μg/L) and the same dynamic range (0.12 μg/L−2.00 μg/L) to that obtained in buffer. These findings indicate that the content of microcystins in river waters can be determined using the standards in PBST. Furthermore, recovery studies were performed by the analysis of twelve river waters spiked at three levels (0.50, 1.00, and 1.50 μg/L). Before spiking, the samples were previously analyzed by LC/MS and with a commercial ELISA test kit. The microcystin content in all samples was below the detection limit of both techniques. As is shown in Table 3, the recovery results ranged between 78% and 113%, allowing the determination of MC-LR in river waters without preconcentration.

The applicability of the microsensor arrays on disk for fast screening of microcystins in river waters is demonstrated. A conventional optical disk drive as detector combined with standard discs as analytical platform can analyze a large number of samples simultaneously in 37 min, what it makes this sensor system interesting, especially in high-throughput screening assays. The good stability of precoated disk and the immobilized immunocomplex indicates the potential of the biosensor for on-site detection and quantification.

The hydrophobic nature of the sensing surface prevents spreading and cross-contamination between samples when dispensing low sample volumes onto the disk. Also, it is worth mentioning that if the array density increases, sample volume could be reduced, and hence, the number of samples could be also easily scaled up. The microsensor disk array, although being very simple in terms of materials, allows reproducible quantitative analysis. The possibility of including external and internal calibration and positive and negative control spots in the same platform simplifies the quantification and improves assay reproducibility.

In terms of sensitivity, the developed methodology performs very well and comparable to other described screening methods for the determination of microcystins (*18*−*22*). Furthermore, unlike the reported assays, the array on disk approach is able to analyze simultaneously a large number of samples, detecting several microcystin congeners in river waters at levels below those guided by national agencies and WHO. The results suggest that the sensor system is a valuable tool for routine analysis of natural and drinking waters for a multitude of targets. In addition, the presented approach combines the best of both the immunoassay and compact disk approaches to yield a sensitive sensing technology that offers the capability of rapid water analysis. In line with this, the described technology suits well with on-site monitoring and in situ analysis demands, especially in relation to the use of low reagent consumption, simple sample preparation, cost-effectiveness, high sensitivity, rapidity, and portability. This finding in combination with the fact that almost all microcystin congeners pose a serious

TABLE 3. Recovery Studies for the Analysis of MC-LR in River Waters

	MC-LR found (μg/L)					
	sample					
MC-LR added (μg/L)	W 1	W 2	W 3	W 4	W 5	W 6
0	<LL	<LL	<LL	0.15 ± 0.01	<LL	<LL
0.50	0.43 ± 0.05[a] (86)	0.51 ± 0.04 (102)	0.39 ± 0.03 (78)	0.56 ± 0.06 (112)	0.48 ± 0.02 (96)	0.48 ± 0.03 (97)
1.00	1.13 ± 0.08 (113)	0.88 ± 0.05 (88)	0.78 ± 0.05 (78)	1.10 ± 0.10 (110)	0.98 ± 0.03 (98)	0.89 ± 0.03 (89)
1.50	1.65 ± 0.12 (110)	1.63 ± 0.10 (96)	1.53 ± 0.09 (102)	1.59 ± 0.12 (106)	1.48 ± 0.15 (99)	1.61 ± 0.12 (107)
	sample					
MC-LR added (μg/L)	W 7	W 8	W 9	W 10	W 11	W 12
0	<LL	<LL	<LL	<LL	<LL	<LL
0.50	0.44 ± 0.02 (88)	0.49 ± 0.03 (99)	0.49 ± 0.04 (98)	0.46 ± 0.03 (93)	0.52 ± 0.03 (104)	0.52 ± 0.05 (104)
1.00	0.83 ± 0.04 (83)	0.91 ± 0.04 (91)	0.88 ± 0.05 (88)	1.05 ± 0.08 (105)	0.99 ± 0.09 (99)	0.79 ± 0.06 (79)
1.50	1.67 ± 0.14 (111)	1.42 ± 0.09 (95)	1.52 ± 0.09 (101)	1.58 ± 0.08 (105)	1.68 ± 0.07 (112)	1.58 ± 0.10 (105)

[a] The figures represent the mean ± SD. The number in brackets is the recovery expressed in percentage. LL: Linearity limit.

human health risk shows that this bioMEM device might be a useful tool for in situ environmental monitoring of microcystins in natural and drinking waters.

Acknowledgments

This work was funded by the Spanish Ministerio de Ciencia e Innovación (MICINN, project CTQ2007-64735-AR07) and by Generalitat Valenciana (ACOMP_GVA-2009/650). The authors thank F. Rubio (Abraxis kits) for kindly providing the coating conjugate and MAb-MC reagents. Also, we would like to thank Lorenzo Monforte from Aguas de Valencia, S.A. to help us in the comparative study between disk and ELISA plate water analysis.

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ES101653R