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Adsorption of Microcystin onto Polymer Covered Gold Chips by Quartz Crystal Microbalance-Dissipation Detection

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Different functional polystyrenes were synthesized and the adsorptions of microcystin-LR onto those resins were monitored by quartz crystal microbalance-dissipation. Both adsorption pH and surface properties had a considerable effect on the adsorption amount, while adsorption temperature was less significant. Ammonium polystyrene would be a better candidate for microcystin-LR adsorption at neutral pH conditions.

Key words: Polystyrene, Quartz crystal microbalance-dissipation, Microcystin-LR, Adsorption

I. INTRODUCTION

Microcystins were first discovered in the 1980s [1] and separated from *Microcystis aeruginosa* [2, 3]. The most widely studied microcystin is microcystin-LR (MC-LR) [4, 5]. Since the presence of microcystins has been documented all over the world [6] and they are dangerous to human health and life [7], there is an urgent need for a method of detoxifying them. To date, many research groups [8–13] have attempted to minimize the dangers of MC-LR. Adsorption has been attempted by both the sediment MMPB (2-methyl-3-4-phenylbutyric acid) method [12] and activated carbon adsorption [8]. Although good effects [14] were observed, some problems remain unsolved. Adsorption is a process that involves the interaction between the adsorbate and adsorbent. Research about effects of adsorbent functional group changes on MC-LR adsorption is limited [15, 16].

Macroporous resins are ideal materials for adsorption, due to their large surface area [17]. In addition, they can be tailored through chemical reactions to satisfy our needs. They are brand new porous polymers developed from ion exchange resins. The latter have a long history in waste water treatment [18], especially for numerous pollutants in ppb or ppm grades. Reports concerning with substances separation [19] and purification [20] using resins have snowballed. Since the surface of macroporous resins can be modulated by chemical reaction, it is possible for us to contrast the differences among different surface groups in the adsorption behavior of MC-LR.

Quartz crystal microbalance-dissipation (QCM-D) is a device using the piezoelectric properties of quartz crystal to monitor the changes in mass occurring on an interface. According to the Sauerbrey equation, the decrease in frequency is proportional to the increase in mass [21]. Many adsorption detection methods are good at testing the amount rather than the mechanism. Quartz crystal microbalance (QCM) is a real time instrument which can monitor the changes during adsorption [22], thus offering us a better understanding of the adsorption processes and mechanisms [23–25].

Polystyrenes functionalized with different groups have never been used to adsorb microcystin. The influence of functional groups on the adsorption of MC-LR onto adsorbent has not been investigated. In this work, we obtained basic data on the adsorption of MC-LR onto the polystyrene resins.

II. EXPERIMENTS

A. Chemicals

Microcystin-LR (>95% purity) was purchased from Express Technology Co., Ltd., Beijing, China and was diluted 100 times with methanol. The stock solution was stored at $-20\text{ }^{\circ}\text{C}$. Other chemicals were purchased from Sinopharm Reagent Company (China, Shanghai) and used as received.

B. Synthesis of polystyrene

In a typical reaction, poly(vinyl alcohol) (PVA) 0.1 g was dissolved in 120 mL water and methylene blue trihydrate 0.1% of 1.0 mL, styrene monomer of 40 g and benzoyl peroxide of 0.8 g were added into the solution

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in sequence. The mixture was heated to 70 °C, with mechanical stirring over 4 h in a three necked round bottom flask, and then maintained at 90 °C for 0.5 h. The particles were isolated by filtering, washing and drying.

C. Synthesis of sulfonate polystyrene

The polymer was synthesized as described previously, with minor modification [26]. In a typical reaction, linear polystyrene of 5.0 g was dissolved in 20 mL cyclohexene and 0.25 mL 98% H₂SO₄ was added stepwise. The solution was heated to 40 °C and maintained for 4 h. In order to neutralize the reaction solution, it was rinsed until being cooled to room temperature and was then distilled in a vacuum to obtain linear sulfonated polystyrene.

D. Synthesis of ammonium polystyrene

Synthesis of chloromethylated polystyrene followed Xu *et al.*'s method [27] with minor modification. Trioxane of 3.6 g and 15.2 mL trimethyl chlorosilane were dissolved in 40 mL chloroform with 4 g linear polystyrene. The solution was cooled to 0 °C and 4.0 mL anhydrous SnCl₄ was added. After agitating the solution at 0 °C for 30 min the mixture was heated to room temperature for 20 min. The reaction was stopped immediately after methanol-water solution was introduced. The supernatant was decanted and the organic layer was washed with water. Methanol was added and distillation was used to obtain linear chloromethylated polystyrene.

The chloromethylated styrene of 1.0 g was dissolved in 15 mL methylene dichloride, agitated and heated to 40 °C with triethylamine 10 mL added dropwise for 3 h. The solution was distilled, washed in methanol-water and dried after synthesis reaction to obtain ammonium polystyrene [28].

E. Characterization of resins

The resins were dried to eliminate all solvent if they were in fluid states. The remaining solid was ground with potassium bromide to obtain about 1 mg mixture. The pressed disc method was then used and the resulting slate was inserted into an infrared spectrum (FTIR) slot for detection. The contents of the functional groups were analyzed by elemental analyzer (vario-MACRO). The contents of sulfur and nitrogen were 4.260% and 1.803% respectively.

F. QCM-D measurement

A Q-sense chamber (Q-sense E4) was used for real-time simultaneous measurement of frequency and dissipation changes.

Au-coated 5 MHz AT-cut quartz crystals were used as the reaction carriers. The Sauerbrey sensitivity of these crystals is 1 Hz=17.7 ng/cm². The frequency and dissipation responses were recorded at 5, 15, 25, 35, 45, 55, and 65 MHz, corresponding to the overtones, $n=1, 3, 5, 7, 9, 11,$ and 13, respectively. For clarity, only the normalized frequency shift ($\Delta f_{\text{normalized}}=f_n/n$) and the dissipation shift ΔD for the third overtone are presented. Since the mass calculation was based on Sauerbrey sensitivity, the obtained mass was Sauerbrey mass considering the contribution of solvents without the influences of viscosity. During the measurements, the crystals were mounted in a thermal static liquid chamber set at 25 or 35 °C to provide a rapid, non-perturbing exchange of the liquid (non-flowing liquid) over one side of the sensor.

The quartz crystal microbalance-dissipation (QCM-D) crystals were first rinsed with solution of 5:1:1 mixture of milli-Q water, ammonia (25%) and hydrogen peroxide (30%) then heated to 75 °C for 5 min. Then the crystals were cleaned with milli-Q water and dried with nitrogen gas. The crystal was then secured on a spin coater (KW-4A, Institute of microelectronics of Chinese Academy of Sciences) under vacuum, and a designated volume of pre-synthesized resins was deposited on the wafer which was then quickly rotated at 2000 r/min for 20 s. The coated film was placed in a 75 °C oven until the membrane was dry.

III. RESULTS AND DISCUSSION

Briefly, the gold chip was first spin coated with polymers of different functional groups and the adsorption of the microcystin onto these surfaces was monitored online in real time. The constitutions of resins were characterized and confirmed by both FTIR and elemental analysis. From elemental analysis, the S and N mass fraction were 4.260% and 1.803%, respectively. The FTIR was then adopted to distinguish the functional groups on modified polystyrene. From Fig.1(b), the peaks at 1194, 1145, and 1005 cm⁻¹ present the stretching vibration of S-O and S=O bonds. The elemental analysis shows a mass content of S to be 4.260% and with simple calculation, the ratio of styrene unit to sulfonate styrene unit is 5.45. In Fig.1(c), the absorption at 1450 cm⁻¹ denotes the bending vibration of aromatic quaternary amine and peaks at 2910 and 2844 cm⁻¹ for asymmetric and symmetric stretching vibration of -CH₃, indicating the existence of quaternary ammonium salt. Based on the structure analysis, the content of N was transferred to direct formula as depicted in Fig.1(c).

After spin-coating of polymer layers onto QCM-D chips, the monitor of adsorption process was processed in real time on instrument. The stock solution was diluted with a mixture of methanol and water at an appropriate ratio. Before the introduction of MC-LR, all tests

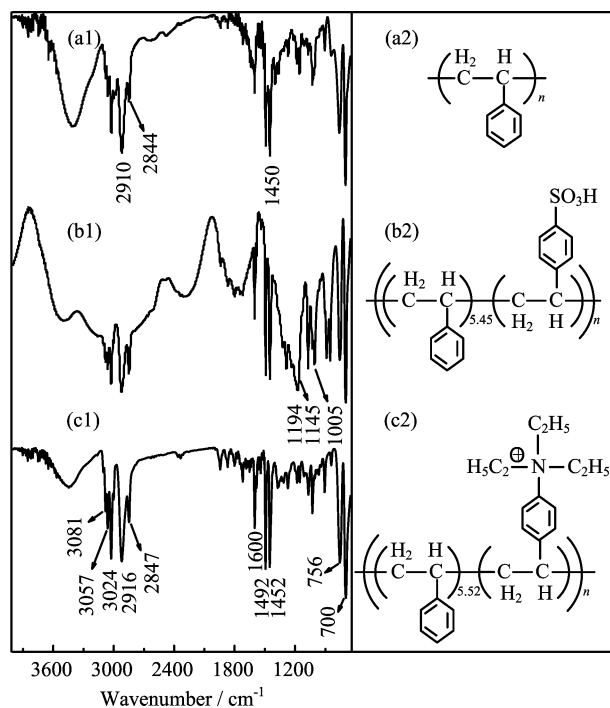


FIG. 1 Infrared spectra and the corresponding structures of (a) polystyrene, (b) sulfonate polystyrene, and (c) ammonium polystyrene. The structures of (b2) and (c2) are calculated from elemental analysis.

begun with flushing buffer solutions to eliminate any interference from solutions. Huang *et al.* [8] hypothesizes that MC-LR is a large molecule with some amino acids which render it hydrophobic. The hydrophobic interaction may make the molecules more prone to adsorbing onto hydrophobic adsorbent's surfaces. However, our results (Fig.2(a), Fig.3(c), and Fig.4(c)) show that at MC-LR concentration of 40 ppb, the frequency changes of polystyrene, sulfonate polystyrene and ammonium polystyrene are -15 , -1.5 , and -120 Hz respectively. As is stated above, the mass adsorbed onto the chips is negatively proportional to the frequency change. Therefore, the more negative the frequency is, the heavier the mass adsorbed. It seems that ammonium polystyrene has an higher adsorption capability than sulfonate polystyrene and polystyrene without modification under neutral conditions. This conclusion runs counter to the hydrophobic interaction theory. In order to reveal the predominant factor influencing the adsorption process of MC-LR onto resins, other factors must also be considered.

The first factor is the solution temperature. Two experiments were conducted at room temperature and 35 °C respectively. Figure 2 shows that temperature has little effect on adsorption, as the frequency difference between these two processes was merely 2 Hz. Since MC-LR is a large molecule, it has many groups in its chain. Among them, one ionizable amino group and two ionizable carboxyl groups principally determine

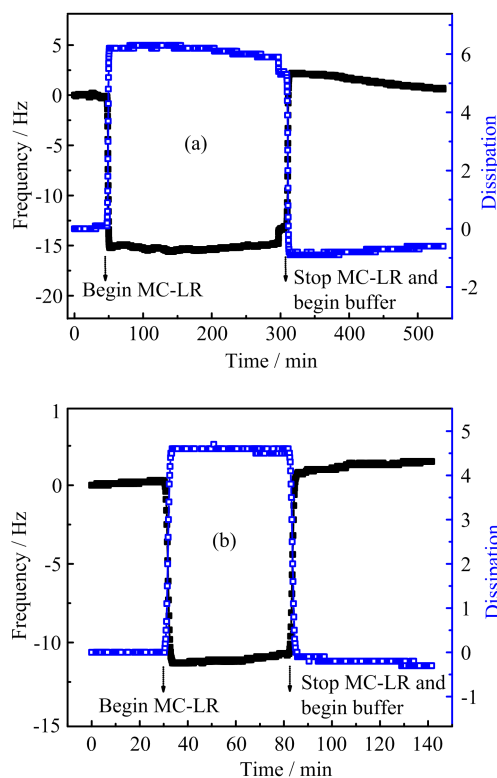


FIG. 2 Adsorption of 40 ppb MC-LR onto polystyrene at different temperature. (a) 25 °C and (b) 35 °C. Open squares are dissipation, solid squares are frequency.

the net charges of MC-LR [29]. The isoelectric points of MC-LR are 2.09–2.19. Therefore, under different pH conditions, the ammonium and sulfonate groups may present different charges. When the pH is between 2.09–2.19, the negative charges are equal to the positive charges, so the net charge is zero and MC-LR is neutral. However, when pH is lower than 2.09, the amount of ammonium is increased, and MC-LR will be presented in a positive state [30]. Therefore, we suppose that electrostatic interaction may play a part in the determination of MC-LR adsorption. The structures of the synthesized functional polystyrene are shown in Fig.1. By changing pH values of the solution, the net charges on MC-LR are controlled. Since the functional groups of resins are ionizable, the effects of electrostatic interactions can be studied.

Figure 3 shows that for sulfonate polystyrene, the adsorption amount of MC-LR is 36.62, 23.30, and 22.49 ng/cm² at lower, medium, and higher pH values when MC-LR presents positive, neutral, and negative in the solution. The masses are calculated according to the Sauerbrey equation,

$$\Delta m = -\frac{S\Delta f}{n} \quad (1)$$

where Δm denotes the adsorbed Sauerbrey mass, n is the overtone number, here $n=3$ for 5 MHz measurements, S denoting the Sauerbrey mass sensitivity is

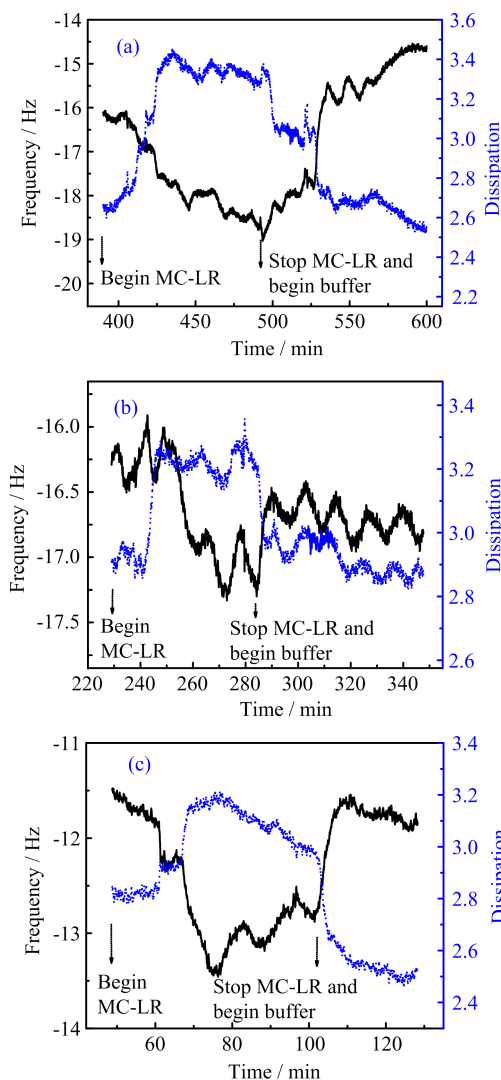


FIG. 3 Adsorption of 40 ppb MC-LR onto sulfonate polystyrene in buffer with different pH values at 25 °C. (a) pH=2.03, (b) pH=2.11, and (c) pH=7.00. Dotted line is dissipation, solid line is frequency.

17.7 (ng/cm²)/Hz [31]. Since the sulfonate group can dissociate two negative charges, when MC-LR bears a positive charge, MC-LR has a greater tendency to combine with negatively charged sulfonate groups on the sulfonate polystyrene. At higher pH values, MC-LR is negatively charged, and the repulsion between MC-LR and negative sulfonate separates the two, thus the adsorption amount is smaller. At medium pH values, MC-LR is neutral. Without a strong attraction or repulsion, the amount of MC-LR which combines with the sulfonate groups lies between the positively- and negatively-charged MC-LR adsorption amounts.

As for the ammonium polystyrene (Fig.4), the adsorption amount increases to 148.88, 150.00, and 2333.40 ng/cm² when pH values are lower, medium, and higher. Ammonium polystyrene has many ammo-

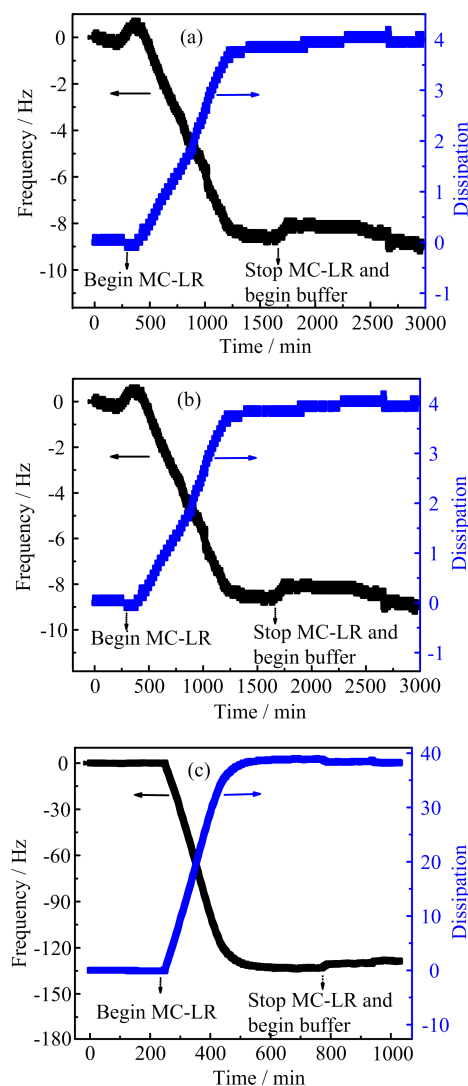


FIG. 4 Adsorption of 40 ppb MC-LR onto ammonium polystyrene in buffer with different pH values at 25 °C. (a) pH=1.77, (b) pH=2.14, and (c) pH=7.00.

nium groups which render it positive in charge and the same charge repels MC-LR and ammonium polystyrene from each other, so there is little adsorption at lower pH values. When MC-LR is neutral, there is no obvious repulsion between the adsorbate and the substrate, and the interaction between them is not strong enough to attach large amounts of adsorbates. Therefore, the adsorption amount under the two pH conditions (lower and medium) is almost identical. However, when the pH value is higher, MC-LRs charge is reversed to a negative value and it therefore attaches to the ammonium substrate. The electrostatic interaction is so strong that ten times more MC-LR is adsorbed.

Aside from the dominant factor in the determination of the adsorption process, another phenomenon is also observed. The traditional method for monitoring adsorption is immersing an adsorbent into adsorbate so-

lutions and detecting the changes in solution concentrations at fixed time intervals. This method is laborious and the mechanism cannot be observed directly. By QCM-D monitoring, not only can the adsorption amount be obtained over fewer time intervals, but also the adsorption mechanism can be clarified.

For polystyrene without modification, the adsorption processes (Fig.2) remain very similar: the frequency rises vertically up to its adsorption peak and balances over time. The overall process for adsorption takes no more than 3 min, which is a rather rapid process. Quick though the process is, adsorption immediately drops when a buffer is introduced, which suggests the interaction between the adsorbate and adsorbent is weak and reversible. We suspect this reversible process can mainly be attributed to the lack of ionizable groups on the polystyrene surface. During adsorption and desorption, the MC-LR on the polymer film shows no structural changes, and its conformation on contact with the film is always the same. It only touches the film surface by simple physical interaction, without further reactions. However, ammonium polystyrene (Fig.4) requires 4 h to 13 h to reach saturation point. When the adsorbent and adsorbate bear opposite charges, the adsorption process is quicker and the adsorption amount is higher. When the two bear the same charge, fewer molecules are attracted and the repulsion interaction slows down the process of saturation. When MC-LR is neutral, bearing an equal amount of positive and negative charges, the data show that the Sauerbrey mass first decreases which suggests some substances desorb from the substrate, and then the adsorption amount increases. Since MC-LR is neutral, it is hydrophobic overall. The hydrophobic interaction is so strong at first that some hydrated water within the membrane is desorbed from the substrate, resulting in Sauerbrey mass loss. When the large molecule reconstructs itself, leaving its functional groups exposed on the exterior, the hydrophilic groups begin to interact with the membrane surface and induce the adsorption.

Since ammonium polystyrene presents the best adsorption outcomes, the effect of MC-LR concentrations on adsorption was also investigated to assess its potential application. The adsorption curve is shown in Fig.5. With the increase of MC-LR, the adsorption onto adsorbent decreased and eventually reached a plateau. Langmuir model was adopted to describe the relationship between adsorption mass (Q) and equilibrium concentration (C). This model assumes identical adsorption sites with one layer molecule adsorption capacity and each site with independent adsorption quantity. The equation is presented as,

$$Q = \frac{Q_{\max}bC}{1 + bC} \quad (2)$$

The parameter b shows the affinity of ammonium polystyrene with MC-LR, and Q_{\max} is the maximum adsorption of MC-LR. After fitting with Lang-

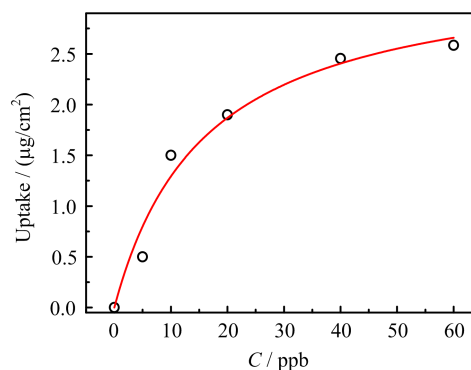


FIG. 5 Adsorption isotherm of MC-LR onto ammonium polystyrene at pH=7.

muir equation, a maximum adsorption capacity of 3370 ng/cm² and the b value of 0.0622 with r^2 of 0.97423 were obtained.

IV. CONCLUSION

Three different kinds of polystyrene were synthesized and applied in the adsorption of MC-LR. The effects of the surface groups changes on adsorption behavior are summarized. From our work, it would be possible to design the surface of adsorption materials with different characteristics. Moreover, adsorption performance for different adsorption materials can also be predicted. Thus, efforts to improve the function and design of adsorbate materials can be greatly assisted by obtaining a detailed understanding of the interactions among different functional polystyrenes and MC-LR. It is expected that this research can provide both practical and fundamental information for more efficient process design, leading to effective removal of MC-LR and improved water quality and safety.

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- [1] D. P. Botes, A. A. Tuinman, P. L. Wessels, C. C. Viljoen, H. Kruger, D. H. Williams, S. Santikarn, R.

- J. Smith, and S. J. Hammond, *J. Chem. Soc., Perkin Trans. I*, 2311 (1984).
- [2] W. W. Carmichael, *J. Appl. Bacteriol.* **72**, 445 (1992).
- [3] R. M. Dawson, *Toxicol.* **36**, 953 (1998).
- [4] C. MacKintosh, K. A. Beattie, S. Klumpp, P. Cohen, and G. A. Codd, *Febs Lett.* **264**, 187 (1990).
- [5] R. Nishiwaki-Matsushima, T. Ohta, S. Nishiwaki, M. Suganuma, K. Kohyama, T. Ishikawa, W. W. Carmichael, and H. Fujiki, *J. Cancer Res. Clin.* **118**, 420 (1992).
- [6] H. W. Paerl, I. I. I. R. S. Fulton, P. H. Moisaner, and J. Dyble, *Sci. World J.* **1**, 76 (2001).
- [7] W. W. Carmichael, *J. Environ. Eng. and Sci.* **3**, 155 (2004).
- [8] W. J. Huang, B. L. Cheng, and Y. L. Cheng, *J. Hazard. Mater.* **141**, 115 (2007).
- [9] B. C. Nicholson, J. Rositano, and M.D. Burch, *Water Res.* **28**, 1297 (1994).
- [10] J. Rositano, B. C. Nicholson, and P. Pieronne, *Ozone-Sci. Eng.* **20**, 223 (1998).
- [11] M. R. Teixeira and M.J. Rosa, *Sep. Purif. Technol.* **46**, 192 (2005).
- [12] K. Tsuji, H. Masui, H. Uemura, Y. Mori, and K. I. Harada, *Toxicol.* **39**, 687 (2001).
- [13] K. Tsuji, T. Watanuki, F. Kondo, M. F. Watanabe, S. Suzuki, H. Nakazawa, M. Suzuki, H. Uchida, and K. I. Harada, *Toxicol.* **33**, 1619 (1995).
- [14] Z. Mohamed, H. El-Sharouny, and W. Ali, *Arch. Environ. Con. Tox.* **52**, 489 (2007).
- [15] R. Considine, R. Denoyel, P. Pendleton, R. Schumann, and S. H. Wong, *Colloids Surf. A* **179**, 271 (2001).
- [16] F. F. Sheng, P. Pendleton, and A. Badalyan, *Int. J. Environ. Tech. Manage.* **10**, 36 (2009).
- [17] J. Germain, J. Hradil, J. M. J. Fréchet, and F. Svec, *Chem. Mater.* **18**, 4430 (2006).
- [18] J. R. Parrish, *Anal. Chem.* **49**, 1189 (1977).
- [19] M. Petro, F. Svec, I. Gitsov, and J. M. J. Fréchet, *Anal. Chem.* **68**, 315 (1996).
- [20] F. Gode and E. Pehlivan, *J. Hazard. Mater.* **100**, 231 (2003).
- [21] G. Sauerbrey, *Zeitschrift für Physik A* **155**, 206 (1959).
- [22] K. Glasmästar, C. Larsson, F. Höök, and B. Kasemo, *J. Colloid Interf. Sci.* **246**, 40 (2002).
- [23] C. Larsson, M. Rodahl, and F. Höök, *Anal. Chem.* **75**, 5080 (2003).
- [24] E. Reimhult, H. Fredrik, and B. Kasemo, *Langmuir* **19**, 1681 (2002).
- [25] R. Richter, A. Mukhopadhyay, and A. Brisson, *Biophys. J.* **85**, 3035 (2003).
- [26] J. C. Yang, M. J. Jablonsky, and J. W. Mays, *Polymer* **43**, 5125 (2002).
- [27] M. C. Xu, Z. Z. Ou, Z. Q. Shi, M. C. Xu, H. T. Li, S. X. Yu, and B. L. He, *React. Funct. Polym.* **48**, 85 (2001).
- [28] X. Chen, Z. M. Chen, Z. C. Cui, K. Zhang, G. Zhang, and B. Yang, *Acta Polym. Sin.* 293 (2003).
- [29] R. P. Schwarzenbach, P. M. Gschwend, and D. M. Imboden, *Environmental Organic Chemistry*, New York: John Wiley & Sons, Inc. Frontmatter, (2005).
- [30] P. G. J. de Maagd, A. J. Hendriks, W. Seinen, and D. T. H. M. Sijm, *Water Res.* **33**, 677 (1999).
- [31] F. Hook, M. Rodahl, B. Kasemo, and P. Brzezinski, *P. Natl. Acad. Sci. USA* **95**, 12271 (1998).