Fresenius J Anal Chem (1999) 364 : 506–511

REVIEW

Franz L. Dickert · Oliver Hayden

Imprinting with sensor development – On the way to synthetic antibodies

Received: 4 November 1998 / Revised: 29 April 1999 / Accepted: 5 May 1999

Abstract Molecular imprinting is an attractive tool for the development of artificial recognition systems. Even non-covalent imprinting provides universal interaction centers for sensoric applications. The coated chemical sensors have high stabilities under harsh conditions in both the gas and liquid phases. With adequate efforts optical and mass-sensitive sensors (quartz crystal microbalance, QCM, surface acoustic wave detector, SAW) are suitable for analysis down to the ppb (nL/L) range. PAHs, isomer VOCs as well as complex oil mixtures are appropriate analytes.

Introduction

The mimicry of biochemical interactions is one of the most challenging questions in various scientific fields. The strategy of molecular imprinting, which has attracted considerable attention in the last two decades, appears to be a promising tool for the development of biochemically analogous recognition systems. This review gives a brief summary of advances in the field of chemosensory devices with non-covalently imprinted coatings.

Nowadays, primarily analytical methodologies use imprinting techniques for the preorganization of molecular recognition sites. Imprinted stationary phases already have reasonable enantio- and stereoselectivity for chromatographical applications for a number of chiral compounds [1–3]. Recently, Wulff et al. [4] reported imprints with substantial catalytic activity. Improved molecularly imprinted coatings for sensor applications are also an intense field of studies. In particular, the detection of small organic molecules will have an impact for future sensor designs [5–7] and molecularly imprinted sorbent assays [8].

The molecularly imprinted polymers (MIPs) are made by polymerization or polycondensation in the presence of a print molecule, thus the polymer skeleton is generated around the future analyte or an analogue template. After polymerization the print molecule is removed by dissolution or evaporation. Figure 1 schematically visualizes the process of imprinting. If the resulting polymer has a robust and porous structure, the complementary binding sites are appropriate for a reversible inclusion process and a selective enrichment of the analyte.

Molecular imprinting can be approached in a covalent and a non-covalent way. The covalent imprinting depends on an easily cleavable arrangement of the template and a monomeric compound, which leads to induced cavities. Non-covalent imprinting refers to a self assembling process by weak intermolecular forces. The latter can be differentiated further into the more distinct hydrogen bond and the weaker van der Waals imprinting. In comparison to the covalent imprinting, non-covalent interactions are not limited to the functionality of the analyte, which makes non-covalent imprinting a more versatile and time-saving technique for a cost-effective development of artificial recognition systems. Most recently, Matsui et al. [9] developed a combinatorial screening system for the preparation of MIPs.

The process of molecular recognition in polymers is still a hot and partially controversial topic [10], but most authors consider a recognition phenomenon, where the selectivity pattern of the MIPs is primarily specified by the imprintable compounds [11], the template [12], the porogen [13] and the polymerization conditons [14]. Contrary to distinct biological affinities, the polymeric receptors have variable affinities, due to the self assembled binding sites. Unspecific condensation-like processes occur on the porous polymer surface and in the narrowing diffusion channels. The advantage of these artificial receptors is their enhanced number of building blocks in comparison to the limited number of amino acids. However, the combinatorial diversity of biological structures is undisputedly out of reach.

Dedicated to Professor Dr. Wilhelm Fresenius on the occasion of his 86th birthday

F. L. Dickert (✉) · O. Hayden
Universität Wien, Institut für Analytische Chemie,
Währingerstraße 38, A-1090 Wien, Austria
e-mail: fdickert@olivin.anc.univie.ac.at

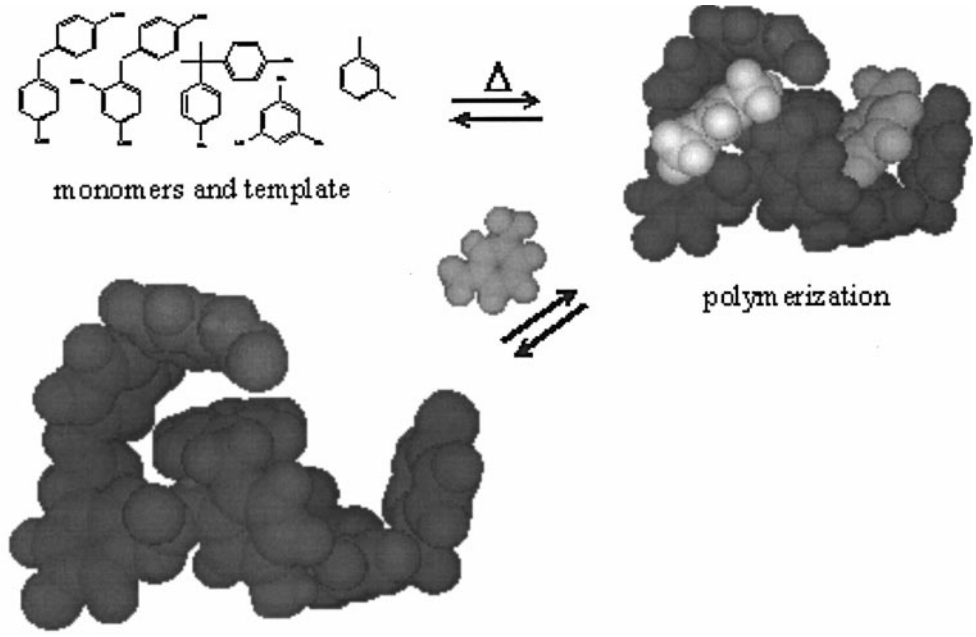

Fig. 1 Non-covalent molecular imprinting with an analyte, which is optionally the porogen

In contrast to thousands of separation plates in chromatography, sensors can only specifically enrich analytes in a single or a few consecutive steps to accomplish an applicable sensor response. Therefore, sensors favorably depend on the bulk effect, whereas chromatographic applications try to avoid bulk phenomena, to prevent peak broadening and tailing. Another methodological difference is the sample preparation. Chromatographic analysis is performed in well defined and often non-aqueous conditions. Chemical sensors, however, are used in variable environments and often in presence of cross-sensitive substances, such as humidity.

The detection of analytes may be performed with not absolutely selective coatings, since condensation occurs under all circumstances. As a result, the sensor response in the gas phase is correlated with the vapor pressure and the molecular weight of the adsorbates. But the coated devices described in this review show the effectiveness of non-covalent imprinting, which still enables selective detections by only minor morphological or functional differences of the analytes. The usage of pattern recognition in field-sensors is a useful tool for a proper evaluation of the sum signals.

Another approach to synthetic antibodies with self-assembling monolayers, which is a field of intense studies, has been proposed by Sagiv et al. [15]. These imprinted monolayers could provide superior response times due to their independence of bulk effects.

Chemical sensors

Sensors of outstanding sensitivity and selectivity still depend on biological recognition systems. But the inadequate stability and reversibility of field-tested biosensors prevent long-term monitoring and applications in harsh conditions. These demands can be met with robust MIP-coated chemical sensors [16].

The versatility of the non-covalent MIPs makes them adaptable to various detection elements, such as chemically modified field effect transistors (CHEMFETs) [17] or liquid chromatography based sensors [18]. Integrated optical [19] and mass-sensitive devices [20], however, promote effective progress in portability and in-the-field-ruggedness with a minimum dependence on changes in the ambient environment. Gravimetric sensors, like quartz crystal microbalances (QCMs) [21] and surface acoustic waves (SAWs), detect mass alterations from the nanogram to the femtogram level, respectively. Figure 2 shows a sensor response of an SAW to ppm (μL/L) concentrations of o-xylene. Considering the lower toxic limit of 100 ppm (μL/L) for xylene, this sensing element would efficiently allow work-place monitoring with reasonable effort. On-chip preparation of the coating and resonance frequencies up to 2.5 GHz emphasize the advantages of these transducers.

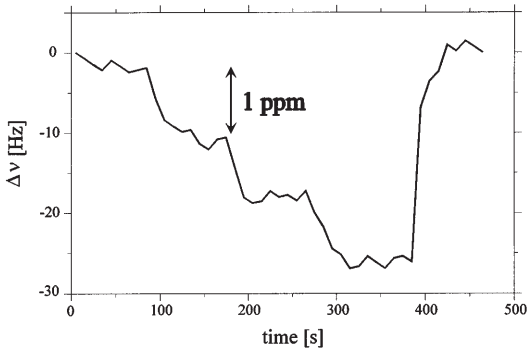

Fig. 2 o-Xylene detection with a coated 433 MHz SAW device

Non-covalent MIPs

The flexible method of non-covalent imprinting has been used for the development of polymerization procedures with accessible compounds, using polystyrene, polyurethane and methacrylate.

If the template is a suitable organic solvent the print molecule itself can act as porogen. This template effect by solvents is often treated as a subset of molecular recognition in the field of organic syntheses [22, 23], where solvent molecules direct a reaction towards the most solvated shape of the product. The removal of the incorporated analytes can be followed spectroscopically, e.g. with the fluorescence activity of incorporated polycyclic aromatic hydrocarbons (PAHs) [24]. In most cases the evaporation of the templates from the polymer matrix is complete, whereas the level of dissolution of less volatile templates varies depending on the layer thickness, the crosslinker amount in the polymer, the solubility and steric properties of the template.

Enhanced selectivity patterns are feasible by covalently embedding monomolecular host molecules into the MIPs. The molecular cavities of these hosts have well-defined preorganized binding sites for the analyte incorporation and act as additional porogen. The tailored monomolecular host molecule binding sites are partially favorable considering the affinity distribution curve of the imprinted sites. The MIP can be interpreted as a preselective barrier previous to the uniform host molecule cavities, leading to a two-step equilibrium for the recognition process.

Evidence for specific inclusion processes

The imprinting effect can be quantified by comparing the sensor response of an imprinted and a non-imprinted layer of equivalent height. Additionally, the BET-model [25] of adsorption and IR-spectroscopy [26] give evidence for intracavitative inclusion processes. The BET adsorption analysis can be performed using the experimental sensor effect data from QCM measurements, since the gravimetric response [19] is correlated to the partial pressure of the analyte. The host-guest interaction in MIPs can also be pursued by NMR [27].

Sensor applications

The detection of vapors with mass-sensitive devices is a well known application. The weak host-guest interactions with volatile organic compounds (VOCs) as templates have been successfully used for the detection of halogenated, polar and aromatic hydrocarbons. Particularly isomer analytes in multicomponent samples represent a challenging task for chemosensory devices.

Different sensor properties can be obtained by simple variation of the amount of crosslinker added (Fig. 3). Ethanol and ethyl acetate have been selected due to their comparable molecular weights and volatility. The highest selectivities and sensitivities are obtained with 10% crosslinker. Lower and higher crosslinker amounts seem to reduce the imprint effect or lead to less accessible binding sites.

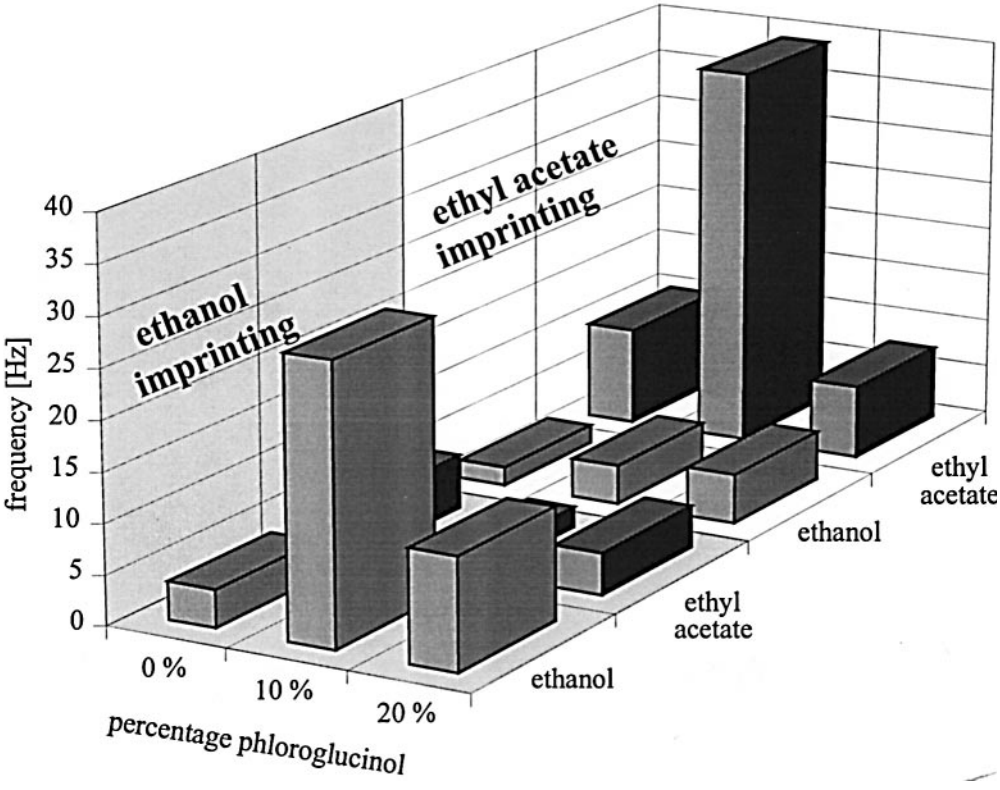

Fig. 3 Polyurethane layers (100 nm) with different amounts of crosslinker and print molecules; sensitivity to 0.1% solvent pulses

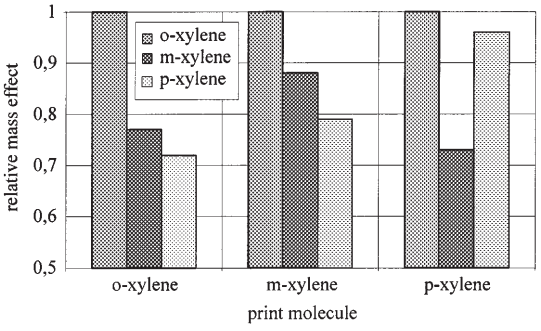

Fig. 4 Sensitivity pattern to xylene pulses of a non-covalent MIP array for the detection of xylene isomers

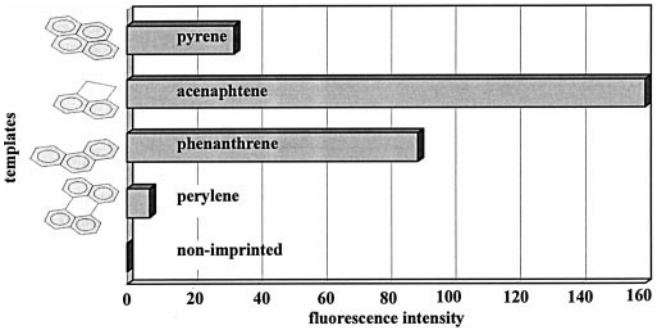

Fig. 5 PAH imprinted coatings with sensitivities in the ng/L range for the detection of pyrene

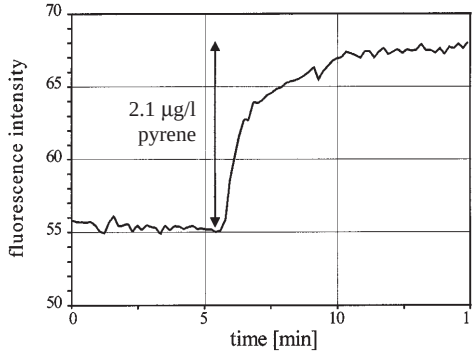

Fig. 6 Fluorescence sensor response to pyrene of a non-covalent MIP coated sensor element

The subtle distinction of nonpolar analyte mixtures in the presence of humidity is probably the most tricky analysis in the gas phase. In Fig. 4 non-covalent MIPs were used for the detection of xylene. Residual humidity effects, which is the major reason for non-linear sensor responses, were corrected by multivariate data analysis. The sensitivity pattern of the QCM sensor array was evaluated with partial least square (PLS) and artificial neural network (ANN) techniques [28, 29]. The backpropagation networks allowed accurate detection of the isomers in the lower ppm range with a root-mean-squared error of prediction of max. 4%. The sensitivity of p- and m-xylene can be inverted by the imprint process, whereas o-xylene, having the lowest volatility, is unaffected and more easily incorporated.

Recently, we have successfully imprinted polymers with PAHs [21]. The analyte binding is a result of efficient π-π interactions between phenylated polymer compounds and the aromatic modules of PAHs. The sensitive layer is highly selective and leads to enrichment factors up to 10^7 from the aqueous phase which is observed with fluorescence detection. In contrast to the imprinted coatings, unspecific PAH adsorption in non-imprinted layers leads to a fluorescence intensity of only 1% of the observed imprint effect. This sensitivity allows detection limits down to 30 fg/g or lower, which is comparable to detection limits in the 10 fg/g range of currently reported immunoaffinity chromatography [30]. The imprinting effect has been amplified using slightly smaller templates in comparison to the actual analyte (Fig. 5); as an example Fig. 6 shows the sensor response to the uptake of pyrene.

Enhanced selectivities have been obtained with covalently embedded host molecules in an imprinted polymer matrix. The covalent embedding is favorable in view of minor water cross-sensitivities and robust coatings for liquid applications. Moreover, crystallization of the macrocyclic host molecules can be prevented. The additional imprinting of the matrix results in adapted diffusion pathways to the imprinted sites and to the "tailored" cavities of the host molecules. This combination of conventional host-guest chemistry with molecular imprinting has been used for the detection of xylene isomers (Fig. 7). Slight alterations of the polymer compounds and different print molecules inverted the xylene selectivities, whereas halogenated or similar aromatic hydrocarbons remained less detectable.

Mass-sensitive transducers can also be used in liquid phases. Although their application in aqueous media is still complicated [31], useful sensors for hydrophobic liquids can be developed. Unspecific viscosity effects can be widely eliminated by a dual arrangement with a second QCM. One example is given for the detection of the degradation process of highly complex motor oil mixtures. Modern high performance oils can consist of a package of additives of up to 30%, which includes various substance classes, for instance detergents, foam suppressors or corrosion inhibitors. The imprinting with a single component is not useful for the monitoring of the overall degradation process. Therefore, imprinting has been performed with the entire complex matrices of fresh and degraded motor oil. These QCM coatings showed constant mass-sensitivities even after a year. In Fig. 8 the imprinting effect is visualized by a change from fresh to waste oil. Obviously, the degraded oil cannot be taken up by the imprinted layer.

A further sensitivity enhancement can be achieved by raising the resonance frequencies of the QCM and SAW resonators, since the signal-to-noise ratio increases in an approximately linear manner with the oscillation frequency [32], whereas the mass resolution depends quadratically on the operating frequency.

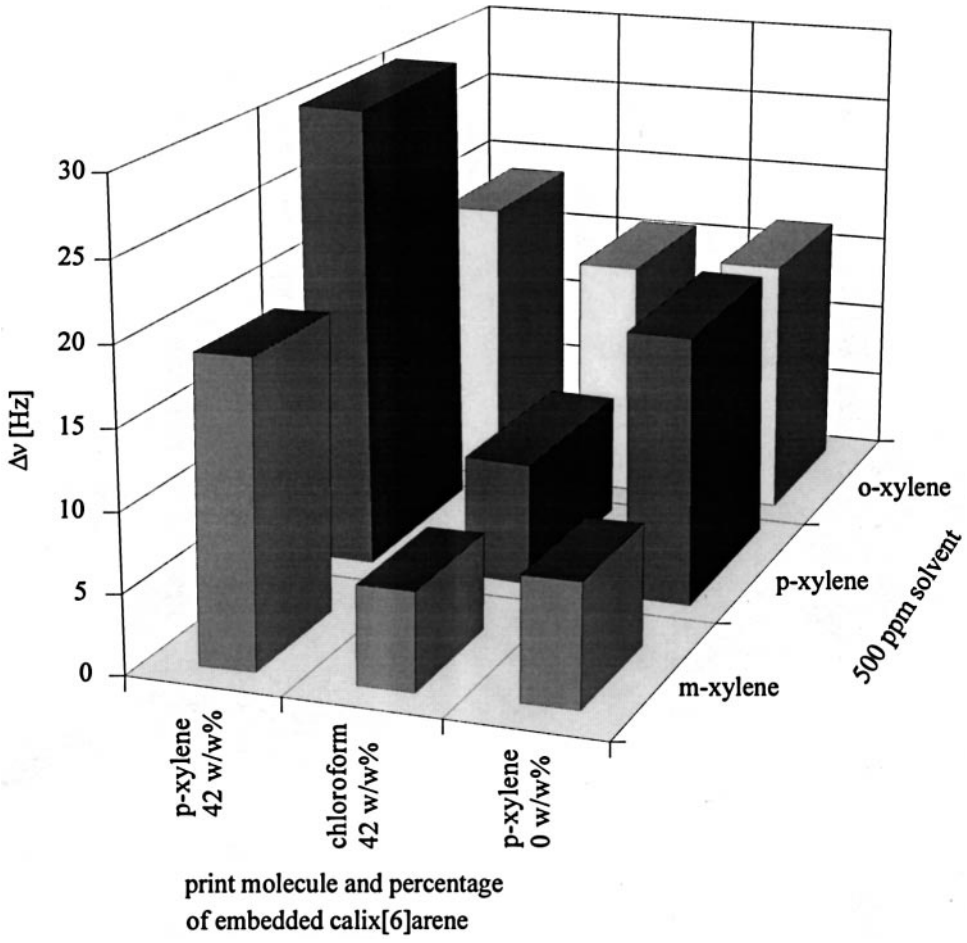

Fig. 7 Detection of 500 ppm (µL/L) xylene isomers with 70 nm polyurethane-coated sensor layers; the coatings are imprinted with p-xylene or chloroform and the percentage of covalently embedded p-t-butylcalix[6]arene varies between 0 and 42 w/w%

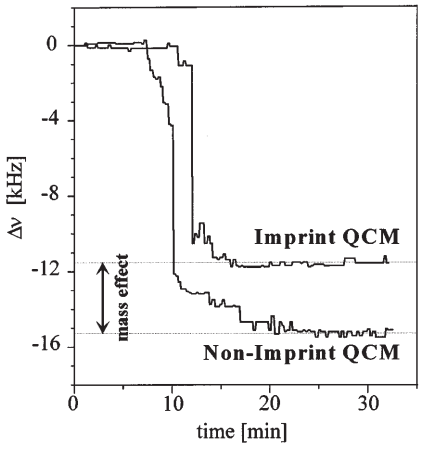

Fig. 8 QCM sensor responses (fresh oil imprinted and non-imprinted coating) in going from fresh to degraded motor oil. In both cases the viscosity effect is equivalent, whereas the mass load of fresh oil in the imprinted layer is lost in the waste oil environment

Conclusion

Hydrogen bond and van der Waals forces enable effective imprinting processes. Besides the main applications in chromatography, non-covalent MIPs offer versatile coatings, particularly in combination with mass-sensitive transducers. The sensitive layers allow the detection of xylene isomer mixtures, PAHs or the monitoring of extremely complex oil mixtures. The ease of preparation combined with their long-term thermal and chemical stability make them suitable for a wide range of applications in gas and liquid phases. Moreover, results are obtained more cheaply and easily than with comparable GC applications.

The polymer receptors can replace less stable biosensors or allow the development of recognition systems for which there are no biological analogues. High-frequency transducers and multivariate data analysis of sensor arrays improve the sensitivity and selectivity pattern in multicomponent samples.

References

1. Yoshikawa M, Fujisawa T, Izumi J, Kitao T, Sakamoto S (1998) Anal Chim Acta 365:59–67
2. Remcho VT, Tan ZJ (1999) Anal Chem 71:248A–255A
3. Zander Å, Findlay P, Renner T, Sellergren B (1998) Anal Chem 70:3304–3314
4. Wulff G, Groß T, Schönfeld R (1997) Angew Chem 109:2050–2052
5. Wessa T, Göpel W (1998) Fresenius J Anal Chem 361:239–245

6. Malitesta C, Losito I, Zambonin PG (1999) Anal Chem 71: 1366–1370
7. Jenkins AL, Uy OM, Murray GM (1999) Anal Chem 71: 373–378
8. Haupt K, Mosbach K (1998) Trends in Biotech 16: 468–475
9. Takeuchi T, Fukuma D, Matsui J (1999) Anal Chem 71: 285–290
10. Grate JW, Patrash SJ, Abraham MH, Du CM (1996) Anal Chem 68: 913–917
11. Sellergren B (1997) Trends in Anal Chem 16: 310–320
12. Piletsky SA, Parhometz YP, Lavryk NV, Panasyuk TL, El'skaya AV (1994) Sensors and Actuators B 18–19: 629–631
13. Yoshizako K, Hosoya K, Iwakoshi Y, Kimata K, Tanaka N (1998) Anal Chem 70: 386–389
14. Sellergren B, Shea KJ (1993) J Chromatogr 635: 31–49
15. Cohen SR, Naaman R, Sagiv J (1988) J Phys Chem 90: 3054
16. Janata J, Josowicz M (1998) Anal Chem 70: 179R–208R
17. Josowicz M, Janata J (1986) Anal Chem 58: 514–517
18. McNiven S, Kato M, Levi R, Yano K, Karube I (1998) Anal Chim Acta 365: 69–74
19. Dickert FL, Thierer S (1996) Adv Mater 8: 987–990
20. Dickert FL, Forth P, Lieberzeit P, Tortschanoff M (1998) Fresenius J Anal Chem 360: 759–762
21. Sauebrey G (1959) Z Physik 155: 206–222
22. Tokunaga Y, Rudkevich DM, Santamaria J, Hilmersson G, Rebek J Jr (1998) Chem Eur J 4: 1449–1457
23. Heinz T, Rudkevich DM, Rebek J Jr (1998) Nature 394: 764–766
24. Dickert FL, Besenböck H, Tortschanoff M (1998) Adv Mater 10: 149–151
25. Dickert FL, Geiger U, Keppler M, Reif M, Bulst W-E, Knauer U, Fischerauer G (1995) Sensors and Actuators B 26–27: 199–202
26. Kobayashi T, Wang HY, Fuji N (1998) Anal Chim Acta 365: 81–88
27. Sellergren B, Lepisto M, Mosbach K (1998) J Am Chem Soc 110: 5853–5860
28. Lavine BK (1998) Anal Chem 70: 209R–228R
29. Dickert FL, Haunschild A, Hofmann P (1994) Fresenius J Anal Chem 350: 577–581
30. Cichna M, Knopp D, Niessner R (1997) Anal Chim Acta 339: 241–250
31. Faraday Discussions (1997) 109
32. Dickert FL, Forth P, Bulst W-E, Fischerauer G, Knauer U (1998) Sensors and Actuators B 46: 120–125