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ORIGINAL PAPER

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# Double molecular imprinting – a new sensor concept for improving selectivity in the detection of polycyclic aromatic hydrocarbons (PAHs) in water

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**Abstract** Highly selective and robust polymer coatings for the detection of polycyclic aromatic hydrocarbons (PAHs) in liquid media have been generated by use of an innovative method of molecular imprinting. By imprinting with two different templates, the selectivity of the polyurethanes used was increased by creating diffusion pathways and molecular cavities. Analyte inclusion was detected both by fluorescence and by use of mass-sensitive transducers. It is possible to optimize layers in respect of the extraction of two different analytes or to achieve extremely high selectivity for a distinct analyte. In this way coatings can be tuned to the lean chrysene, e.g., and it is enriched by a factor of approximately fifty compared with the more quadratic pyrene with the same number of aromatic rings. Measurements of PAHs in water were also performed with a quartz crystal microbalance, which shows that humic acids are not incorporated by the layers and thus do not influence the fluorescence properties of the layers.

## Introduction

Polycyclic aromatic hydrocarbons (PAHs) are carcinogenic substances formed in incomplete combustion processes, e.g. in diesel engines [1]. They are emitted to the atmosphere adsorbed on the surface of soot particles and are then deposited into ground and surface water. Because of their harmful properties, the detection of PAHs is an important field in environmental analysis. PAHs can be detected with fluorescence spectroscopy in the visible range [2, 3, 4, 5]. In direct measurements in liquid media, however, fluorescence yields can be reduced by the presence of quenchers such as humic acids, which are often to be found in surface-water samples.

To enhance the sensitivity and selectivity of fluorescence measurements and continuously monitor PAH concentrations in water, sensitive layers can be used which selectively extract only the analyte from the sample [6]. In this way quenching by humic acids can be eliminated. By combining these layers with fiber-optics [7, 8, 9, 10, 11] or mass-sensitive transducers, highly selective sensor systems can be produced. Because PAHs have no pronounced functionality, the development of sensitive layers focuses on molecular imprinting techniques [12, 13, 14]. Analyte molecules can be added to a solution of monomers, which are then polymerized directly on the transducer forming a stable layer. After washing out the template, cavities remain in the polymer which are optimized to a distinct analyte molecule. By variation of polymerization conditions such as temperature, the amount of crosslinker used, and the template, these polymers can be adapted to almost any application. A further advantage of molecular imprinting is that the polymers generated have excellent stability in aqueous media, are insoluble in organic solvents, and are chemically inert. The concept of double imprinting introduces an additional degree of freedom in material design, because combinations of two different printing molecules enable the creation of more suitable hollows for analyte re-inclusion and improved diffusion pathways. In this technique one template molecule acts as a porogen.

## Experimental

*Chemicals.* Bisphenol A (2,2-bis(4-hydroxyphenyl)propane, >97%) and *p,p*'-diisocyanatodiphenylmethane (60%, containing 5% of other diisocyanates and 30% of triisocyanates) were purchased from Merck. Phloroglucinol (98%), toluene (p.a.), tetrahydrofuran (THF, p.a., <0,05% $H_2O$), and humic acid (pract., ~20% ignition residue) were purchased from Fluka. The PAHs used were benzanthracene, chrysene, perylene (all Sigma, 95%), acenaphthene (Sigma, 99%), pyrene (Fluka, >97%), and naphthalene (Fluka, 99%). All reagents were used as received.

Dedicated to the memory of Professor Dr. J.F.K. Huber

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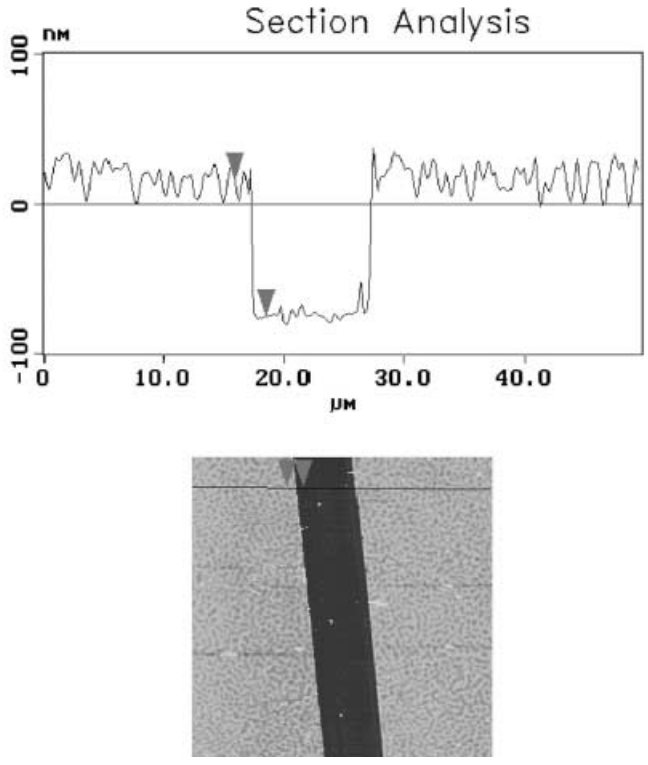


**Fig. 1** Cross-section and height image of a polyurethane layer spin-coated on to a glass substrate at 2000 r.p.m., as recorded with an atomic force microscope in tapping mode. The thickness of the layer is 94 nm and its roughness is only slightly greater than that of the substrate

*Preparation of sensor layers.* For measurements of PAHs in aqueous solutions, polyurethanes were prepared from bisphenol A and *p,p*'-diisocyanatodiphenylmethane with phloroglucinol as an additional cross-linker. The ratio of isocyanate to phenol groups was 5:9; phloroglucinol accounted for one third of the total amount of phenol groups. The monomers were dissolved in THF so as to furnish a total concentration of 20 mg of monomers $L^{-1}$. Templates were added in an amount equivalent to 5% of the mass of monomers. As shown in previous work [15], concentrations higher than 5% lead to the formation of excimers during fluorescence spectroscopy, because the PAHs are incorporated as clusters. In double-imprinted layers both templates together amounted to 5% of the mass of monomers. The layers were prepared by spin-coating the solutions on to quartz or glass substrates at 2000 r.p.m. The layers were then polymerized for 12 h at temperatures between 20°C and 70°C.

This procedure results in homogeneous layers with a reproducible thickness of 90–100 nm and a surface roughness of approximately 15 nm, which is only slightly larger than that of the substrate, as can be seen in Fig. 1. The topographic image was recorded using a Digital Instruments Nanoscope III atomic force microscope operated in tapping mode. A cross section is also shown; this reveals the layer thickness to be 94 nm. After polymerization, the imprint was washed from the layers by immersion in toluene for 2 min.

*Measurements.* After drying, the layers were placed in a flow of PAH-containing water. PAH-solutions were produced by sonication of a defined amount of PAH in water for 30 min and subsequent dilution to the desired concentration. The concentration of PAH in the solutions was directly validated by fluorescence measurement. Between preparation and use the solutions were stored in dark flasks. To characterize the extraction efficiency, the ratios of the concentrations of the PAH in the layer and the solution – the enrichment factor, analogous to Nernst's distribution law – were calculated [15]. Already published results [16] show that an imprinted layer yields a nearly linear sensor characteristic for, e.g., pyrene in the range 30 ng $L^{-1}$ to 40 µg $L^{-1}$, whereas at higher concentrations saturation behaviour is observed. All measurements were performed in the linear ranges. The enrichment factors are directly related to the slope of the linear range. Thus, typical concentrations of the PAHs were several µg $L^{-1}$, depending on their solubility in water. The concentration of PAH in the layers was determined by comparison of the fluorescence intensity with that from a reference layer containing 5% of the respective PAH. The reference layers were produced in the same way as the sensor layers, only substituting the 5% imprint with the desired analyte. The method is only valid if the fluorescence yield does not change with increasing PAH concentration in the layer. This was confirmed by performing mass-sensitive measurements [15]. Fluorescence measurements were performed with a Perkin–Elmer LS50B luminescence spectrometer.

## Results and discussion

Numerous changes in selectivity were observed when the template and the polymerization conditions were varied; some highlights are presented. Figure 2 shows the selectivity achieved with a 25% naphthalene–75% perylene (of the total 5% imprint) imprinted layer polymerized at 45°C. Enrichment of chrysene is almost twenty times larger than that of benzanthracene and exceeds that of pyrene fifty times, despite the fact that all PAHs have the same number of benzene rings.

In Fig. 3 the selectivity pattern of three layers with different imprints is shown. The anthracene-imprinted layer results in maximum enrichment for anthracene and the chrysene-imprinted layer for chrysene. A double-imprinted layer prepared with 50% anthracene and 50% chrysene is sensitive toward both analytes. The absolute values of the enrichment factor are lower than those of the single imprinted layers, because only half the amount of either template was used. In Figs 2 and 3 the influence of polymer-

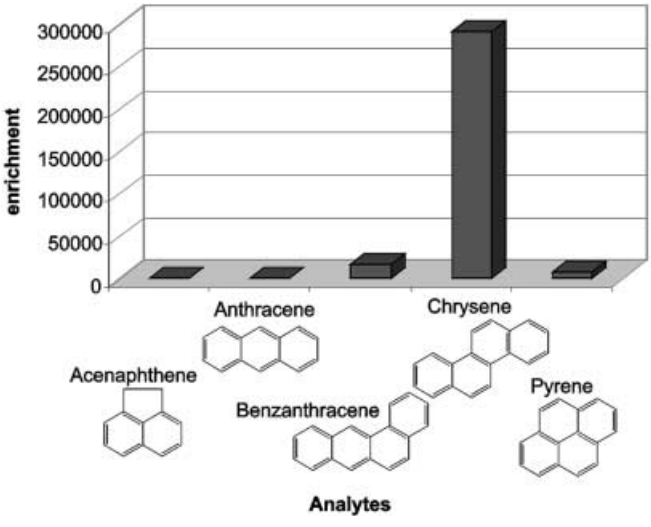


**Fig. 2** Enrichment of different analytes by a polyurethane imprinted with 5% w/w of PAHs (25% naphthalene and 75% perylene) and polymerized at 45°C

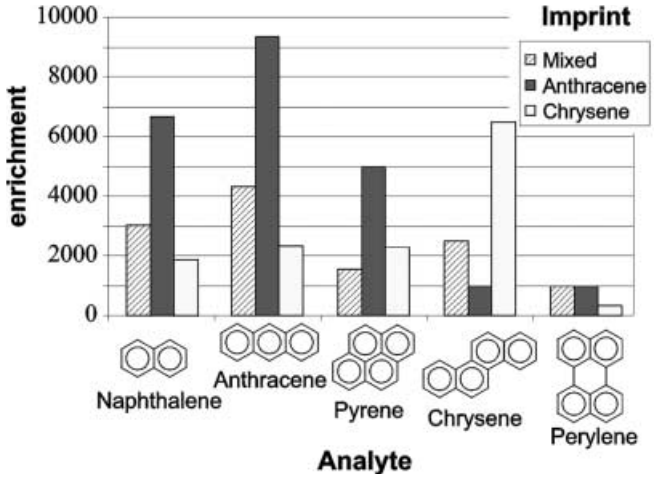


**Fig. 3** Comparison of the selectivity of two single imprinted layers (anthracene and chrysene) and a double-imprinted layer imprinted (anthracene–chrysene, 1:1), the double-imprinted layer enables enrichment for two analytes

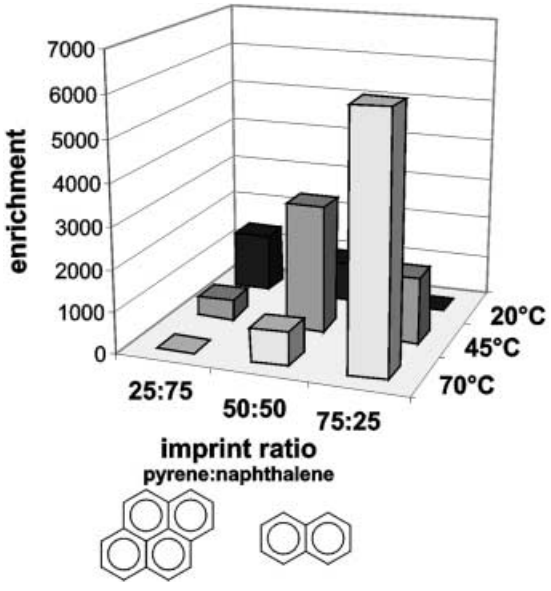


**Fig. 4** Sensitivity of different double-imprinted layers to anthracene, showing the influence of temperature and imprint ratios

ization conditions on the selectivity pattern is impressively demonstrated, resulting either in highly selective layers that are tuned to one analyte only or layers that can detect the sum of two analytes, albeit with smaller responses. This possibility is particularly promising for environmental analysis, because knowledge of the total contamination with different PAHs is often desirable.

The influence of polymerization temperature on the sensitivity pattern towards anthracene is shown in Fig. 4; different amounts of pyrene and naphthalene were combined for the printing process. At 20°C the best enrichment is obtained with 25% pyrene and 75% naphthalene (of the total 5% imprint), whereas this maximum shifts towards larger percentages of pyrene with increasing temperature. This behaviour indicates that the polymer matrix is more closely arranged around the template molecules at higher temperatures, leading to cavities which are more appropriate for the template. At 20°C more of the smaller naphthalene imprint is needed to create cavities for the inclusion of anthracene. A possible explanation would be that optimized cavities are created by naphthalene with the same short axis as anthracene, whereas larger amounts of the more bulky pyrene would generate suitable diffusion pathways.

In this concept, double imprinting enables optimized tuning of cavity size, because it can be more precisely modeled by a large and a small imprint molecule than only by one type. Furthermore, one of the template molecules, obviously the larger, assists as a porogen the creation of diffusion pathways and thus the analytes can reach the interior of the molecular hollows. In addition to this improved tuning, more efficient extraction can be achieved by polymerizing at higher temperatures. The maximum enrichment at 70°C is more than four times as large as that achieved at 20°C. The tight fit of the polymer matrix around the imprint at higher temperatures leads to a more efficient analyte extraction.

All layers previously mentioned were polymerized in air. Experiments were also performed with polymerization in a THF-saturated atmosphere. The enrichment of pyrene for acenaphthene and pyrene single imprinted layers, polymerized both in air and in THF-saturated air, is shown in Fig. 5. Polymerization in a solvent-saturated atmosphere has the same effect as increasing the temperature. When polymerized in air, the layer imprinted with the smaller acenaphthene enables better enrichment of pyrene, whereas the pyrene-imprinted layer is better when a THF-saturated atmosphere is used. As mentioned in the experimental section, the solvent used for preparation of the polyurethanes is THF. Polymerization in a THF-saturated atmosphere prevents rapid evaporation of the solvent from the layers, thus maintaining a lower coating viscosity during the polymerization process. This lower viscosity leads to increased mobility of the oligomers during polymerization, which in turn leads to a tighter fit around the templates.

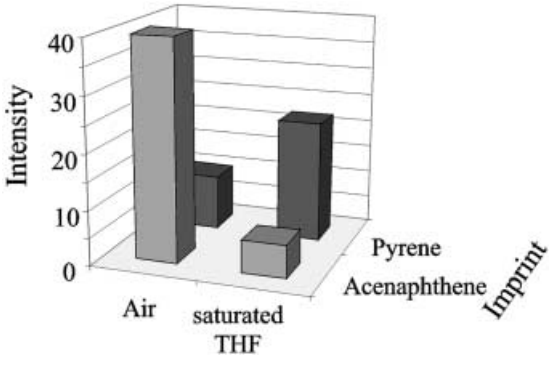


**Fig. 5** Dependence on the surrounding atmosphere of the sensitivity of pyrene- and acenaphthene-imprinted layers to pyrene

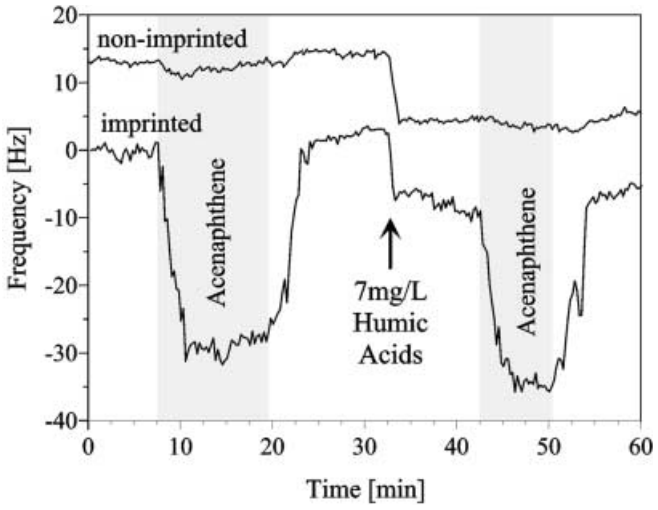


**Fig. 6** QCM frequency response of an imprinted and a non-imprinted polyurethane to solutions containing acenaphthene (5 µg $L^{-1}$) and humic acids

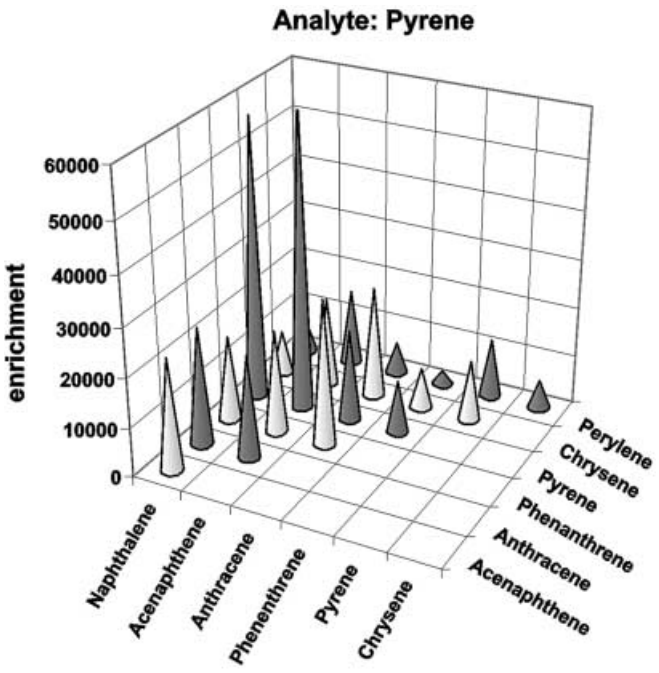


**Fig. 7** Overview of the pyrene enrichment of different double-imprinted layers

As already mentioned, humic acids act as quenchers in fluorescence measurements of PAHs. Using a sensor layer helps to eliminate this unwanted cross effect. The decrease in fluorescence intensity when using a sensor layer was only 8% for 14 mg $L^{-1}$ humic acids, compared with 58% when fluorescence was measured directly in the solution [15]. The influence of humic acids in the PAH solution was also tested by using a two-channel quartz crystal microbalance (QCM) in a flow-cell. The frequency response of the QCM to changes in PAH concentration is shown in Fig. 6. Two pulses of acenaphthene are shown, one without and one with 7 mg $L^{-1}$ humic acids added to the solution. A small instantaneous step can be seen between the pulses in both the imprinted and the non-imprinted layers; this is caused by the change in the conductivity of the solution, because of the humic acids. This physical effect of changes in conductivity is identical for imprinted and non-imprinted layers and can, therefore, be eliminated by differential measurements. The frequency response to acenaphthene in the imprinted layer is the same, irrespective of the concentration of humic acids. By calculating the difference between the imprinted and the non-imprinted channel, it is possible to measure the PAH concentration. Even if the concentration of humic acids is increased by a factor of three no significant interference was found.

In Fig. 7 the enrichment of pyrene is plotted against a variety of templates used for double imprinting. Each layer has a characteristic sensitivity to each PAH; this is indicative of selective behaviour. The sensitivity data in Fig. 7 show the best result for pyrene detection which was achieved by variation of the temperature and of the template ratio. Layers imprinted with naphthalene–pyrene and acenaphthene–pyrene afford excellent enrichment of approximately 60,000 compared with data ranging from 5000 to 20,000 for the other combinations. When compared with the best pyrene single-imprinted layer – an enrichment of 30,000 at a polymerization temperature of 70°C – this means sensitivity enhancement by a factor of two. This shows that double imprinting is a very effective method for creating robust sensor layers with excellent selectivity and sensitivity by creating both optimized molecular cavities and diffusion pathways.

## Conclusion

Molecular imprinting provides manifold possibilities for optimization of sensor layers, e.g. temperature, the templates, and the solvent system. This guarantees more refined adaptation of sensitive layers to specific analytical problems. With double molecular imprinting it is not only possible to create highly sensitive coatings with enrichment factors of up to 300,000 for a specific analyte – layers sensitive to two different analytes can also be created. Fluorescence measurements yield detection limits down to approximately 30 ng $L^{-1}$ [15, 16] and a dynamic range of several orders of magnitude [16] can be realized. Thus nearly every desired sensitivity and selectivity pattern can be generated.

Mass-sensitive measurements were performed which show the excellent reversibility and reproducibility of analyte inclusion irrespective of the concentration of humic acids. The results presented here demonstrate the extended usefulness and the wide range of possible applications of the new technology of double molecular imprinting.

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## References


1. Miguel AH, Kirchstetter TW, Harley RA, Hering SV (1998) Environ Sci Technol 32:450–455
2. Cañizares P, Luque de Castro MD (1996) Fresenius J Anal Chem 354:291–295
3. Kumke MU, Löhmannröben HG, Roch T (1995) J Fluoresc 5: 139–152
4. Panne U, Lewitzka F, Niessner R (1992) Analusis 20:522–542
5. Wolfbeis OS (1985) Trends Anal Chem 4:184–188
6. Muldoon MT, Stanker LH (1997) Anal Chem 69:803–808
7. Brecht A, Gauglitz G (1997) Sens Actuators B B38:1–7
8. Hurum DC, Wandruszka R, Grey AE (1991) Anal Lett 24: 905–911
9. Arnold MA (1992) Anal Chem 64:1015–1025
10. Taib MN, Narayanaswamy R (1995) Analyst 120:1617–1625
11. Alarie JP, Bowyer JR, Sepaniak MJ, Hoyt AM, Vo-Dinh T (1990) Anal Chim Acta 236:237–244
12. Mayes AG, Mosbach K (1997) Trends Anal Chem 16:321–332
13. Wulff G (1995) Angew Chem Int Ed Engl 34:1812–1833
14. Dickert FL, Thierer S (1996) Adv Mater 8:978–990
15. Dickert FL, Tortschanoff M, Bulst WE, Fischerauer G (1999) Anal Chem 71:4559–4563
16. Dickert FL, Besenböck H, Tortschanoff M (1998) Adv Mat 10:149–151