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Sensor Materials
- Detecting Molecules, Mixtures and Microorganisms -

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ABSTRACT

Sensor materials based on molecularly imprinted organic and inorganic polymers were designed and characterized according to their selectivity and sensitivity using mass-sensitive quartz crystal microbalances (QCMs). Cavities of differing shapes and sizes were created in both organic and inorganic polymers, able to selectively re-include the template species. Imprinting was performed both on the molecular and the micrometer scale. The chemical and biomimetic sensors developed allow the detection of various analytes, such as VOCs in ambient conditions, complex mixtures (automotive engine oils) and microorganisms. Thus, the extraordinary flexibility of templating methods is proved as the most versatile platform technology for advanced sensor materials.

INTRODUCTION

The design of chemical sensors is a key issue in modern analytical chemistry, as they open us the way for small, easy-to-use and economically priced analytical instruments. Sensors usually consist of a chemically sensitive layer, a transducer and an electronic data collection and processing system. The keynote is the design of materials with pronounced chemical recognition abilities. The idea is to design sensor layers with antibody-like selectivities, but without the disadvantages of biological materials, such as biodegradation, availability, costs and - most important - ruggedness against physical and chemical stress [1].

Early strategies for layer development included host-guest interactions with molecular cavities, such as crown ethers, cyclodextrins or calixarenes [2]. Although these materials have proven to be highly suitable for the specific detection of small organic molecules, major improvements in sensor layer design can be achieved by molecular imprinting methods [3,4]. In this case the later analyte or analyte-analogue species is used as template and mixed with the precursors of a highly cross-linked polymer or ceramic material. The template molecules are removed from the material after polymerization, either by evaporation or washing. The resulting cavities and diffusion pathways are corresponding to the respective analyte and hence ideally prone to re-inclusion. Some of the major advantages of chemical sensor design by molecularly imprinted layers are the following: the resulting polymer is usually chemically and mechanically stable, can be produced by well-established technological processes (on-chip polymerization or spin coating), and no costly chemicals are needed. Additionally, it could be shown that templating materials impose no limits concerning analyte size, shape and chemical composition. In the present study sensor materials for the detection and monitoring of different sizes, shapes and chemical composition were designed. Analytes surveyed include VOCs and automotive engine oils as well as microorganisms.

EXPERIMENTAL DETAILS

Sensor Layers. Sol-gel layer synthesis was accomplished by acidic hydrolysis of the respective precursor. To produce silicon sol-gel materials 50 μ l tetraethoxysilane 50 μ l diethylaminopropyltrimethoxysilane, 20mg capric acid as template were mixed with 50 μ l water in 900 μ l ethanol. After pre-polymerizing the mixture for two hours at 70°C and three days at room temperature, the resulting sol was spun onto the gold electrodes of a QCM.

Titanates had to be pre-hydrolysed with concentrated hydrochloric acid before carrying out the polymerization reaction. Therefore 100mg tetraethoxytitanate and 6 μ l concentrated hydrochloric acid were heated at 70°C for 1h and then left hydrolysing for 2 days. Then 30 mg of capric acid, 20 μ l of water and 1,2 ml ethanol were added and the mixture was heated again for 1 hour at 70°C. After 3 days of condensation the sols were ready for spin coating. Non imprinted materials were prepared in the similar way in the absence of capric acid.

Pre-polyaddition of the polyurethanes was performed in tetrahydrofuran (THF) with functionality ratios of isocyanato-groups : hydroxy-groups = 1:2. Reactive 4,4'-diisocyanatodiphenylmethane (30% trisocyanato monomers, technical grade) was used for the polyurethanes and bisphenol A as a donor of hydroxy-groups. The polymerisation was continued under stirring conditions at 70°C to reach gel point. For the coating process pre-polymer solutions were diluted 1:100 with THF. *S. cerevisiae* with a moisture content of 30% was used for the imprinting of polymers.

Chemicals and microorganisms. The reagents were used as received from MERCK and FLUKA. We used lyophilized *E. coli*, active dry *S. diastaticus*, compressed and cultivated *S. cerevisiae*. *E. coli* strain W (ATCC9637) was a product of SIGMA. *S. cerevisiae* from ANKER was in a compressed form. Cultivation of yeast was done in universal growth medium (YPD) for 24h. The yeasts were washed from growth medium and centrifugated. Yeasts and bacteria were resuspended in a 1/15 molar $\text{KH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ buffer of pH 6. Cell concentrations were determined using a Neubauer improved erythrometer. The freshly prepared cell suspensions for the measurements showed no significant agglomeration of microorganisms (controls performed with light microscopy).

Yeast stamp. The stamp was prepared by flattening compressed *S. cerevisiae* yeast between a glass slide and Teflon. Yeast cells adhered on the glass slide and could easily be lifted off from the Teflon surface.

QCM Devices. QCM microbalances were produced by screen-printing the desired electrode structure on a quartz blank. Therefore, AT-cut blanks were used because of their favorable temperature coefficient. The thickness of the electrodes produced is about 200 to 300 nm and the surface roughness is below 10 nm.

Frequency measurements. Microbalances were driven with self-produced oscillator circuits. Frequencies were measured with a Keithley 775 A frequency counter. Computational data acquisition was done via a HP-IB bus and a self-programmed software. For gas measurements, streams of defined gas and humidity content were produced by Tylan mass flow controllers, in liquid phase self-constructed measuring cells were used.

DISCUSSION

Detection of organic solvent vapors

When preparing a chemically sensitive layer for a definite analyte, a wide variety of parameters has to be taken into account. Figure 1 shows the effect of different titanate sol-gel layers exposed to butanol vapor. Capric acid was used in all samples as a template to produce molecular hollows. The titanate layers synthesized only differ by the precursor used - ethoxy (TBT) or butoxy group (TEOT). Both the magnitude of the sensor responses and the time constants are significantly changing. Although all layers consist of a pure Ti-O-network, only the sensor coated with the material resulting from the butyl precursor shows pronounced sensor effects. The response times increase with titanate layers in going from TBT to TEOT. The layers have exactly the same stoichiometric composition (no alkoxy groups left in the layers). The number of sites capable of re-including butanol differs due to different layer porosities. The sensitivity of non-imprinted layers is nearly negligible.

Pronounced selectivities of sensor materials can be achieved by molecular imprinting. Figure 2 shows the sensor response to octane vapors of a titanate layer from TBT imprinted with capric acid. N-octane is incorporated into the material preferably compared to the iso-octane. The capric acid as template leads to the formation of elongated, linearly shaped pores in the ceramic material. These cavities are suitable for the incorporation of the slim-shaped n-alkane, whereas

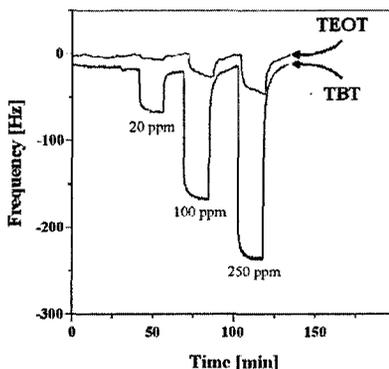


Figure 1. Sensor effects of titanate sol-gel layers synthesized from tetraethoxy-titanate (TEOT) and tetrabutoxy-titanate (TBT) towards butanol.

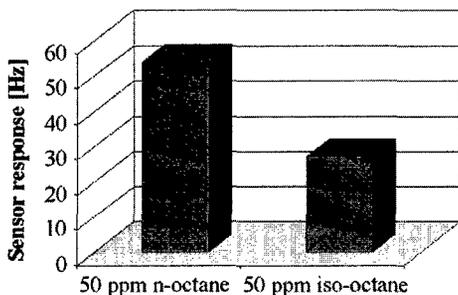


Figure 2. Sensor response of a titanate layer imprinted with capric acid towards differently branched analytes of the same molar mass.

the branched iso-octane is too bulky to fit efficiently into the cavities. Thus, the molecular sieve-like titanate sensor layers allow to selectively distinguish between isomeric analytes. The template directed synthesis of "porous" sol-gel layers is believed to be an economical approach to generate very stable interaction sites for analytes.

When measuring a series of different n-alkanes with the same concentration the frequency changes are following a quadratic relationship to the molecular weight. All these data can be used to model sensor effects for the system under observation. Generally, predictions of the sensor responses are not possible using solely the molecular mass of analytes, as this does not take into account the shape of a given molecule. Excellent results are obtained, however, by including sterical features into the calculations according to the so-called Wiener Index, which can be calculated as shown by the following formula:

$$w = \sum_{i=1}^{n-1} \sum_{j=i+1}^n b_{ij} \quad (1)$$

Where: n number of knots in the molecule
 b_{ij} length of the shortest distance between the knots i and j

In this way well established correlations to the boiling points of linear and branched alkanes [5] can be observed and therefore this idea should also be a suitable tool for this investigation. In Figure 3 the sensor effects obtained for different compounds are plotted against their respective Wiener Index. Once again a nearly quadratic relationship can be observed. Nonetheless there is a fundamental difference between this curve and the observation mentioned above: the data set shown in the figure also contains branched molecules, which also perfectly fit into the model curve. Therefore a topological model for the prediction of the sensor effect caused by a defined compound could be found. This is a highly advantageous tool for the assessment of observable frequency shifts.

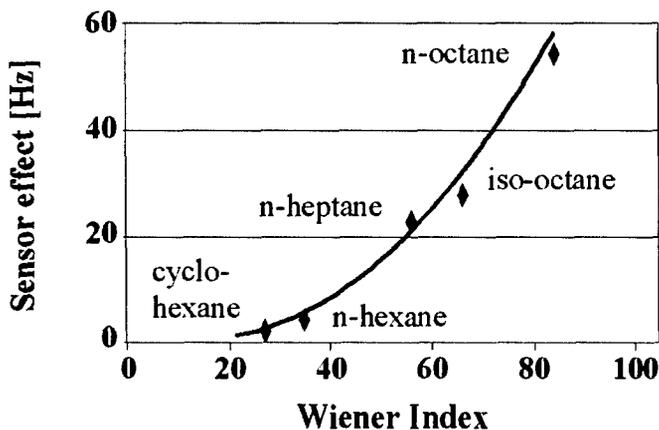


Figure 3. Correlation between the sensor effects of different organic compounds and their respective Wiener Index.

Engine oil degradation measurements

Molecular imprinting has given extraordinary flexibility to sensor layer design. This enables the chemist to develop chemically sensitive materials for analytes which before were beyond the reach of mass-sensitive detection principles. One strategy makes use of the fact that imprinting does not necessarily need an exactly defined template compound. We apply this fact to design a sensor system suitable for the monitoring of automotive engine oil degradation. Such sensor systems would be highly appreciated commercially, as the determination of the remaining useful time for a lubricant is not only of economical but also of high ecological interest. Both organic and inorganic polymers were used for imprinting, we also employed different templating strategies. First, polyurethanes were imprinted with fresh and used engine oils, respectively, the resulting layers yielded mass-effects according to their template molecule [6]. Naturally, engine oils are highly complex mixtures of a base oil and an additive package. Nonetheless molecular hollows can be generated in a polymer thus leading to a functional material capable of selective lubricant inclusion. Contrary to methods used up to now for engine oil characterization, which usually depend on a single chemical (total acid number, total base number) or physical (viscosity, conductivity) parameter, the mass sensitive sensor monitors changes in the entire chemical composition of the lubricant. This means that a totality of chemical changes is transferred into a change of frequency, or, in other words, a very complex procedure can be monitored by a comparably easy measurable quantity. To corroborate the theory of a mass-effect resulting from adapted cavities due to molecular imprinting, series of measurements were carried out with lubricants of different defined states of degradation and the resulting frequency shifts were correlated with one of the physical parameters mentioned above, the so-called Total Base Number (TBN), which is decreasing with the time of use (see Figure 4). The pure mass effects (viscosity changes are already compensated) give an excellent correlation and a good dynamic range not only with increasing oil age but also with TBN. Thus the sensor signal proves its reliability for the monitoring of degradation processes. In contrast to the classical determination

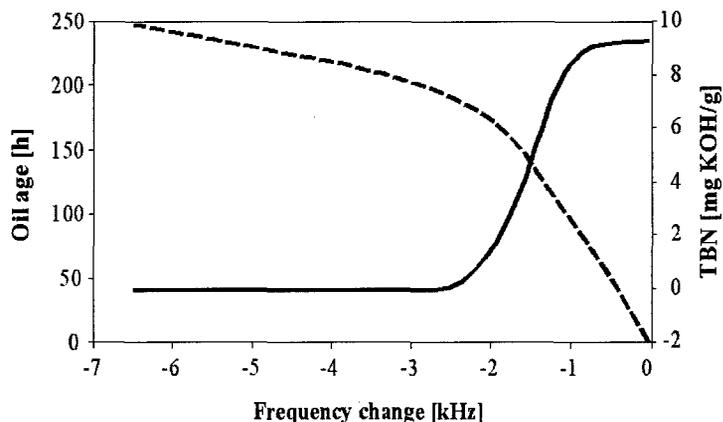


Figure 4. Correlation of the sensor responses to the oil age (---) and Total Base Number (TBN) - a classical oil quality parameter.

of lead compounds, the sensor signals are influenced by the whole complex chemistry changes in the matrix rather than only by one parameter and the oil quality can be assessed directly on-line and does not need to be determined in a laboratory.

Inorganic polymers are also highly suitable for the design of chemically sensitive materials for the monitoring of engine oil degradation. Sol-gel glasses based both on silica and titanate materials have been tested for this purpose. Owing to the fact that these materials are synthesized in aqueous alcoholic (i. e. highly polar) media it is not possible to use the entire engine oil as template due to solubility problems. Hence a lead component has to be determined to fulfill the experimental needs. This can be done best by regarding the effects that occur when a lubricant is degraded: additives are worn out and base oil components get oxidized increasingly, until organic acids are formed (which in fact is measured when determining the TAN of an oil). So it was decided to use a carboxylic acid as template which combines an aliphatic residue of reasonable length with a suitable solubility. As a result of these prerequisites capric acid was chosen to produce an imprint layer for used oil. Additionally, diethyl-aminopropyl-trimethoxysilane was added to the precursor mixture to achieve not only sterically fitting cavities but also to make use of chemical interactions between these basic groups in the material and acidic components in the oil. Figure 5 shows the mass effects of such a layer on the change from a fresh oil to a lubricant worn for 248 hours in a test stand (which, of course, can be regarded as being

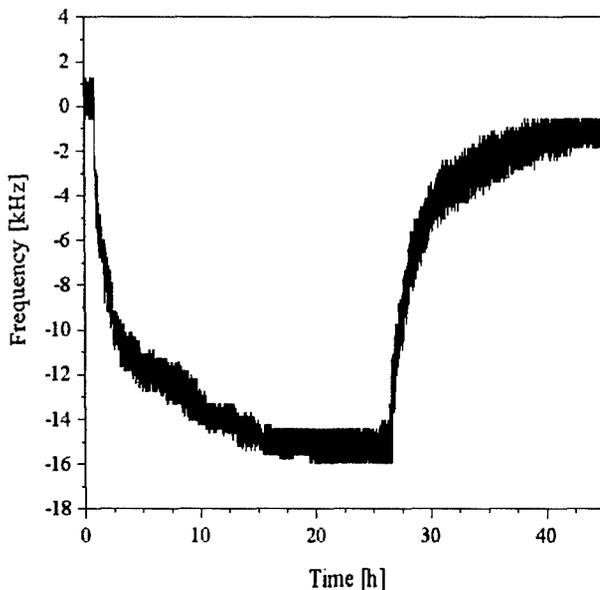


Figure 5. Mass effect of a capric acid imprinted sol-gel layer consisting of tetraethoxysilane and diethylaminopropyl-triethoxysilane (sensor in fresh oil, at 0 h change to waste oil, at 27 h change to fresh oil – proving reversibility)

completely exhausted) and back. The signal was obtained by a differential measurement between an electrode coated with an imprinted layer and a reference electrode and does therefore not contain any influence of changing viscosity. It is obvious that the material incorporates used engine oil preferably to the corresponding fresh lubricant thus yielding a reliable method for oil analysis. The resulting mass effect of 16 kHz is very high and has about the same magnitude than the corresponding frequency shift caused by the change in viscosity. This proportion is much higher than the previously observed ones. It also proves the preferential incorporation of used oil into the sensing layer and this technique proposed might be suitable for the purpose desired.

The next step in the design of mechanically, chemically and thermally stable sensor materials for engine oils is the development of purely inorganic recognition films, i.e. polymers, which do not have any organic substituents left in the sequence. In this case the materials investigated were not restricted to silicon-containing sol-gel glasses, but also other elements show favorable properties. In commercial sol-gel-methods especially alkoxides of transition metals such as aluminum and titanium are widely used (e.g. for the production of soft contact lenses). We prepared titanium glasses imprinted with capric acid, which also showed pronounced re-inclusion effects towards used engine oil. Although layer synthesis requires more experience and time, these materials show some highly favorable qualities: most of all, it is comparably easy to produce homogeneous layers on the respective device, but also the resulting layers are very stable and hard and therefore highly suitable for the rugged environment of a car engine.

The quartz crystal microbalance is highly suitable for engine oil deterioration measurements directly in the liquid phase. Additionally, gas phase would also be an interesting task, as no viscous interaction should occur between the surrounding medium and the device. Of course, some facts have to be taken care of: in order to avoid splash of small droplets to the

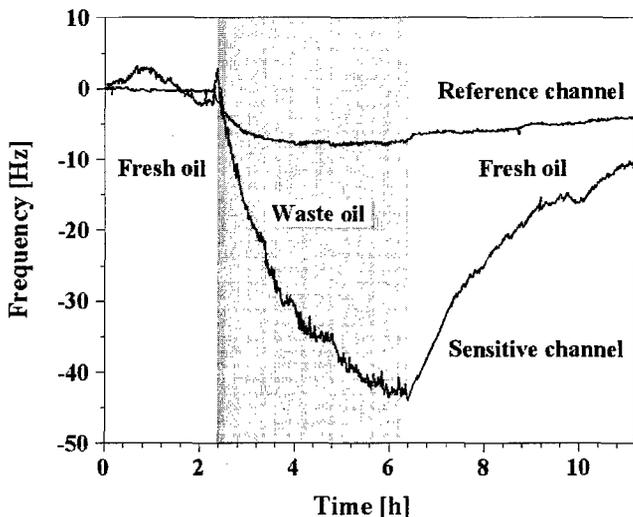


Figure 6. Sensor effects obtained with heatable QCM and an imprinted titanate layer in the gas phase.

sensor surface, which would add a major viscosity contribution to the mass effects observed, the entire device had to be encapsulated somehow. Furthermore, it has to be taken care that no oil vapors condense on the quartz surfaces and thus a heated device was used. Figure 6 shows the sensor response of both the coated and the uncoated channel of a QCM. While the uncoated channel scarcely shows any response the resonance frequency of the imprinted QCM decreases. Again, this observation can be traced back to the incorporation of waste oil components into the sensitive layer.

Microorganism detection

Diffusion limitations occur for the enrichment of the analyte in the bulk of the sensor layer, when going from small organic molecules to biopolymers or even whole cells. Surface imprinting strategies are a favorable way to overcome the limitations in the bulk. However, the number of imprinted cavities in the bulk outnumbers the imprinted pits on the surface by far. Dense receptor sites on the layer surface are needed to obtain a good sensitivity comparable to the results with "bulky" MIP sensor layers.

We are particularly interested in forming sensor layers on-chip, to demonstrate industrial production capability of our sensor design [7]. We therefore developed an elegant technique to form packed receptor sites on dual microbalances coated with a polymer surface using a stamping procedure as depicted in Figure 7. Polymer or sol-gel solutions in a pre-polymerized stage are spin cast onto the transducer surface. A stamp with a cell layer is pressed on the coated device and will embed the cells partially, depending on the layer thickness and the viscosity of the pre-polymer. Receptor sites are built during the curing process due to the self-organization process of oligomeric components around the cell surface. During this polymerization step primarily crosslinking and densification of the pre-polymerized layer will occur. Best imprinting results were gathered with constant force applied on the stamp during the polymerization of

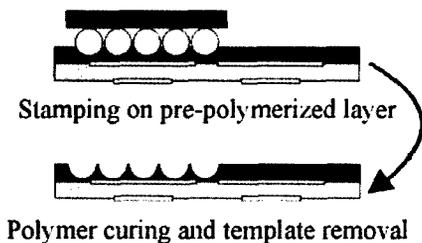


Figure 7. Schematic figure of the surface imprinting process using a stamp with a cell lawn.



Figure 8. Yeast imprints on a polyurethane layer.

polymers. In the case of the sol-gel layers, the best results were achieved pressing the stamp on the coated microbalance for a short time. However, QCMs coated with yeast imprinted polymers (yeast-IPs) from sol-gels are much less sensitive than MIPs from polyurethane. The imprinted pits are densely packed and form a honeycomb-like structure on the polymer surface. The imprints do not have uniform diameters but a size distribution corresponding to the cell population used for the stamp. In Figure 8 the fingerprint of templating yeast cells on polyurethane is shown.

The residual moisture of the cell is a critical parameter for surface imprinting. A water content too high inhibits proper polymerization around the cell and only flattish imprinted sites are molded. On the other hand, too low moisture contents will result in molds with smaller diameters than needed for the average cell size. In the case of yeasts, we chose compressed yeast as template, which has a moisture content of around 30% or freshly cultivated yeasts which have been washed and centrifuged on filter membranes. With these templates the average mold diameter is sufficient to have a large geometrical fit between sensor layer and adhered cells. The templating cells are removed with hot water after curing. Imprinted polyurethane layers e.g. are highly adhesive to the sensor devices and hot water creeps in-between the stamp and the sensor layer. The cells are resuspended and the stamp is lifted-off. A short washing step in an ultrasonic bath for some seconds usually removes even deeply embedded cells from the polymer layer. We have not been observing problems concerning the destruction of the surface MIPs due to the removal of the stamp. The yeast-IPs of polyurethane have pits with a usual depth of up to 1 μm . Therefore, the sensor layers have to be thicker than 1 μm to get a complete fingerprint of the cells – our layers were around 2 μm .

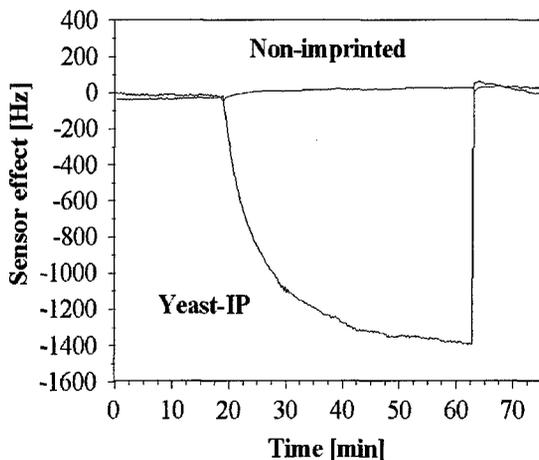


Figure 9. Polyurethane coated dual QCM. Sensor effects due to a buffered suspension (pH 6, 5 ml/min, 20°C) of 1 mg/ml *S. diastaticus* on a yeast-IP (sensitive channel) and non-imprinted polymer layer (reference channel).

We applied the layers on dual QCMs with an imprinted channel and a non-imprinted reference electrode [8]. Figure 9 shows a typical sensor response on the sensitive and reference channels. With this approach we are able to study specific and unspecific adhesion online. We also can perform excellent compensation measurements on dual QCMs. The compensation of QCM measurements in aqueous phase is done favorably on a single quartz disc. In this way, the same temperature dependence characteristics are guaranteed and the close distance of both electrodes is ideal for compensating viscosity and pressure fluctuations during online measurements. No sensor effect is observed on the non-imprinted polymer (reference channel). To apply layers with a micrometer sized "roughness" to QCMs is unusual. These conditions could lead to a non-Sauerbrey behavior, since the adhered yeast cells are comparable to a viscoelastic layer. However, we could show that the sensor is highly sensitive to yeast with a performance equal to natural antibodies applied to QCMs observing typical gravimetric responses. The sensor effects are totally reversed by simply increasing the flow rate to 100 ml/min pulses. The extensively increased shear forces drag the cells out of the pits. However, flow rates in the range of 1-10 ml/min for example do not effect the sensitivity (liquid cell volume is around 100 μ l). Interestingly, in streaming conditions no sensor effect occurs in presence of smaller microorganism, such as "sticky" E.coli, which can adhere in the larger pits. Using light microscopy we could see that the yeast cells with typical diameters of \sim 5 μ m roll over the sensor surface and adhere from time to time in appropriate cell-fingerprints

CONCLUSION

The extraordinary variety of sensing applications of molecularly imprinted polymers could be tested and proven. Potential application can take place in gaseous as well as in liquid phase, the dimension of analytes can reach from uniform molecules such as organic solvent vapors over complex mixtures (e.g. engine oils) to microorganisms with a size up to a few micrometers. An elegant technique for bioimprinting of sensor layers was described. This new type of robust cell sensor is capable of being used for biomass control in bioreactors, since the polymer is autoclavable. The surface imprinting concept is supposed to be an effective platform technique for the imprinting of microorganisms, viruses and enzymes.

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