Title

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Journal

Macromolecular Symposia, 291-292(1)

ISSN

1022-1360

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Publication Date

2010-05-01

DOI

10.1002/masy.201050534

Amphiphilic Polymer Conetworks as Matrices for Phase Transfer Reactions

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Summary: Amphiphilic polymer conetworks were prepared, characterized and used as highly activating matrices for phase transfer reactions. Several applications such as biocatalysis in organic solvents, metathesis reactions in water, biosensor designs for detecting metabolites in organic solvents, as well as gas sensor designs are discussed.

Keywords: atomic force microscopy; biocatalysis; biosensor; gas sensor; polymer networks; radical polymerization

Introduction

Amphiphilic polymer conetworks (APCNs) consist of two immiscible polymers covalently bound to each other (cf. top left of Figure 1). Since the parallel introduction of APCNs by the groups of Kennedy[1] and Stadler,[2] many different synthetic approaches towards APCNs have been described.[3] A common synthetic way is still the protecting group strategy by Ivan and Kennedy,[4] but the in-situ crosslinking of polymers derived by living polymerization is a growing field, particularly performed in the group of Patrickios.[5] In all cases, the incompatibility of the polymers affords a phase separation. However, the covalent linkage prevents the formation of macroscopic areas and well ordered domains in the size of several nanometers (typically 5–25 nm) are observed. The existence of such morphologies has been proven by AFM, TEM, NMR, SAXS and SANS measurements.[6–9] The morphology and size of the domains are dependent on the way of crosslinking, the length and the flexibility of the polymer segments. APCNs with similar volume fractions of the incompatible polymers form a morphology that can be considered as a perturbed lamellar structure. The results are two more or less regularly interconnected nanophases, i.e. the APCN contains two independently acting polymer fractions on the nanoscale (cf. Figure 1, top right). In fact, this morphology is more similar to the gyroid than the lamellar morphology. Although the gyroid morphology appears only in a narrow compositional range in microphase-separated linear block copolymers, it is noteworthy that this morphology occurs over a wide composition window in APCNs.

For microphase-separated APCNs of asymmetric composition, two possibilities exist for the minority phase. One is that the minority phase is embedded into the matrix of the majority phase, as with linear block copolymers of similar composition (Figure 1, bottom left). The other possibility is that the two phases are interconnected even at relatively high compositional asymmetries (Figure 1, bottom right).

Although many applications have been found for APCNs, including controlled drug release,[10,11] tissue engineering,[12,13] pervaporation membranes,[14] extraction of water from organic solvents,[15] and shape memory materials,[16] the only application that truly uses the properties of both interconnected phases are soft contact lenses.[17,18]

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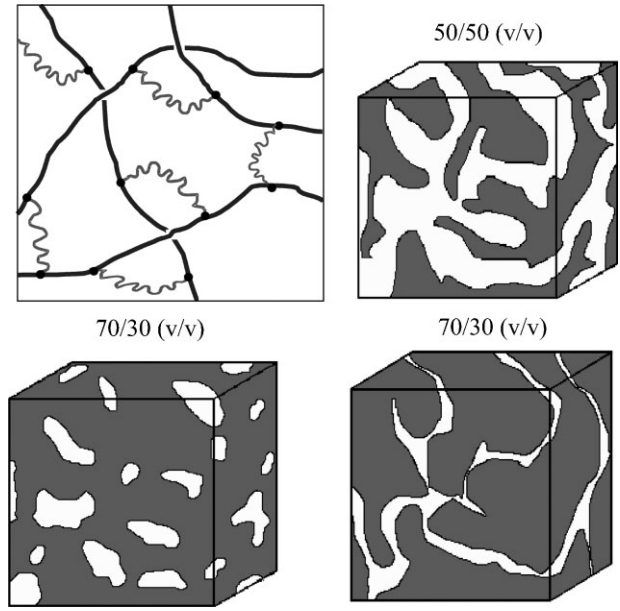


Figure 1.
Topology (top left) and morphologies of APCNs. The APCN composition is indicated above each morphology.

The morphology scenario when both polymer phases are fully interconnected over a broad range of compositions is particularly suited for applications that can not be found for common polymer matrices. Here, the whole matrix shows the properties of two different polymer phases in the dry as well as in the swollen state and both phases are fully and independently accessible. Such a material is the perfect basis for phase transfer reactions, which are typically performed in a solvent mixture. The disadvantage of the latter media is that they are often hard to separate and therefore difficult to handle. The APCNs might provide themselves to overcome this problem.

In order to approach such an application using an APCN, the hydrophilic phase can be loaded with a catalyst that is not soluble in, e.g., an organic solvent. Upon drying, the catalyst is dispersed in the matrix. A possible substrate dissolved in the respective solvent can then diffuse into the matrix and will be transferred to the huge interface. In this contribution, the properties of APCNs as matrices for such phase transfer reactions will be discussed on several examples covering metal catalysis, biocatalysis, biosensors, and gas sensors.

Results and Discussion

Synthesis and Characterization

In this work, APCNs based on poly(dimethylsiloxane) (PDMS) or perfluoropolyethers (PFPE) and different acrylates were synthesized, characterized and used for the various applications. In order to prepare the APCNs, we chose the general synthetic route developed by Iván and Kennedy.[4] In this approach, a polymer with two polymerizable end-groups (macrocrosslinker) is copolymerized with different monomers, which are hydrophobized with the cleavable trimethylsilane (TMS) protection group. The specific synthetic route used here is shown in Figure 2. A great variety of acrylates with different functional groups can be introduced as hydrophilic phase, which is advantageous compared to polymer analog conversions.[19,20] One downside of the concept is the relatively slow conversion

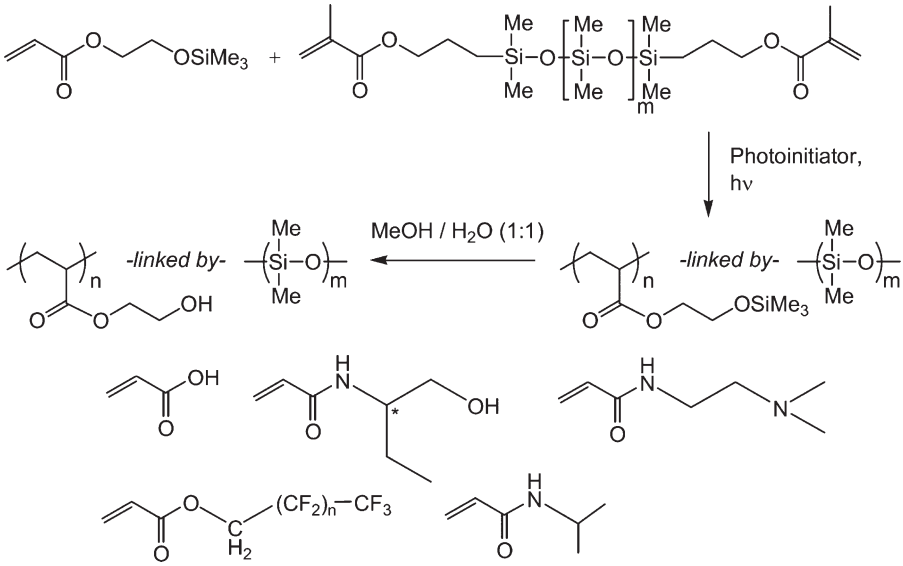


Figure 2.
General synthetic route for the preparation of APCNs by the copolymerization of various acrylates with PDMS dimethacrylate crosslinker.

of the double bonds when using thermal polymerization conditions for the free radical copolymerization. This allows the polymers to separate prior reaching the gel point and will eventually lead to a material that is covered with one of the two polymers in the reaction mixture. In order to avoid early separation, we chose to use photoinitiated free radical copolymerization, because the gel point is reached much faster compared to the thermal polymerization. Figure 3 shows the setup for such an experiment.

In the setup, the polymerization takes place between two glass slides. If a covalently attached film is needed, one glass slide is modified with acrylate groups and the other is covered with a non-adhesive PP-tape. If a free standing membrane is targeted, both slides are covered with the PP film. First, the synthesis was carried out by copolymerizing 2-(trimethylsilyloxy)-ethyl acrylate with a PDMS crosslinker. After photopolymerization, the trimethylsilyl (TMS) group was removed using a 1:1 mixture of methanol/water to give a copolymer that contains poly(2-hydroxyethyl acrylate) (PHEA) linked by PDMS (PHEA-*l*-PDMS). The quantitative cleavage was monitored using ATR-FTIR. The films were then characterized by atomic force microscopy (AFM) applying the phase mode, which allows to distinguish between hard and soft areas on the nanoscale. The surfaces as well as the bulk (images of cryocuts) of these films were

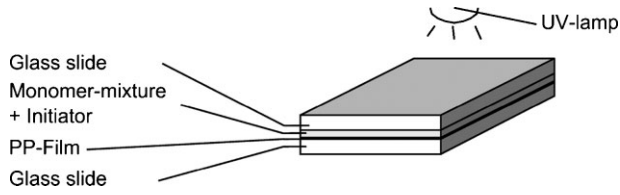


Figure 3.
Experimental setup for the APCN synthesis.

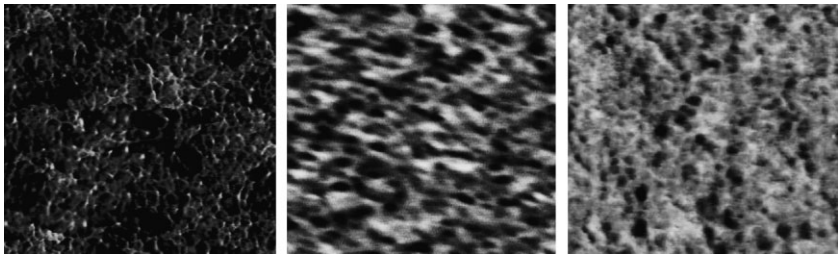


Figure 4.
AFM images (phase mode) of the bulk of PHEA-*l*-PDMS APCNs, containing 70 wt% (left), 50 wt% (middle), and 23 wt% (right) of PDMS. All images have a size of $500 \times 500\ nm^2$. The dark phase corresponds to PDMS in all cases. The M_n of PDMS is 5200 g mol^{-1} ($M_W/M_n = 1.3$).

explored this way. Figure 4 depicts the AFM images of the bulk of PHEA-*l*-PDMS APCNs with different compositions showing the soft PDMS phase dark and the hard PHEA phase bright. Interestingly, all three predicted scenaria could be found (cf. Figure 1 in the Introduction).

As seen in Figure 4, the APCN with 50 wt% PDMS shows the typical interconnected phases as described above. Increasing the PHEA content to 77 wt% results in PDMS spheres embedded in a PHEA matrix. In contrast, a conetwork with 70 wt% PDMS shows a morphology, where the PDMS is the matrix that contains a thin interconnected PHEA network. Thus, as shown in the example of PHEA-*l*-PDMS, both predicted morphologies can be found in APCNs. The synthesized conetworks swell in water as well as in organic solvents. Varying the acrylates resulted in numerous novel conetworks containing a PHEA phase,[8] a poly(acrylic acid) phase,[21] a fluorophilic phase,[22] a chiral phase,[23] and a cationic phase.[24] The synthesis of hydrophilic/fluorophilic APCNs was successfully carried out by copolymerizing perfluoropolyethers with methacrylate end-groups with the perfluorosilyl-modified 2-hydroxyethyl acrylate as described above and subsequently cleaving the silyl groups.[22] These APCNs swell in water and even in perfluorinated solvents as well as in supercritical CO_2.

Alternatively to films and membranes, the APCNs can be prepared as nanostructured microparticles using suspension polymerization. The resulting particles are some 20 to 40 μm in diameter and fully nanostructured as shown with AFM on the surface as well as on silicone-embedded cryocuts.[25]

Biocatalysis

Biocatalysis is a booming field in organic chemistry, because enzymes are the most active, regio- and enantioselective catalysts known. Due to modern biotechnology, they can be purchased at price ranges that compete with those of metal organo catalysts. However, the reaction medium for enzymes is water, and proteins are not soluble in most organic solvents. This fact limits the number of applications of the biocatalysts in organic media. It is noteworthy, however, that Klibanov and others showed in the 1970s and 1980s that enzymes are still able to work in organic media, presenting there a number of unusual reactions, e.g., inversed reactions of hydrolysis and oxidation reactions with inversed enantioselectivity.[26] One important downside of working with enzymes in organic media is that they are often 6 to 7 orders of magnitude less active than the biocatalyst in water. Although numerous approaches have led to enhanced enzyme activities in organic solvents, there is still not enough activity for the industrial breakthrough.

APCNs are excellent candidates for activating enzymes in organic solvents. The concept is illustrated in Figure 5. First,

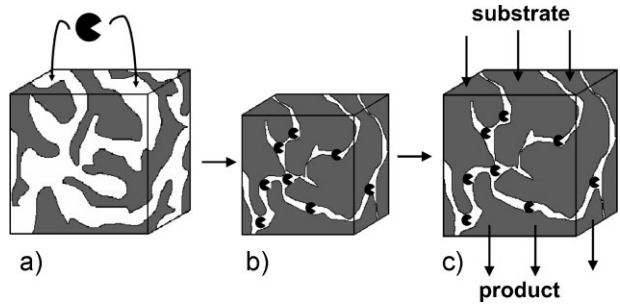


Figure 5.
Concept of activating enzymes in APCNs.

the enzyme can be loaded into the hydrophilic phase by soaking it in an aqueous solution of the protein (Figure 5a). Upon drying, the enzyme should be molecularly dispersed in the hydrophobic matrix (Figure 5b). Introducing the enzyme-loaded APCN in a reaction mixture containing an organic solvent, the reactants will diffuse into the matrix, and then enzymatically be converted at the huge interface of the network, and the formed products will easily diffuse into the surroundings (Figure 5c). This technique allows a milder immobilization of enzymes compared to the common covalent immobilization.[27,28]

It was found in the example of the fluorescently-labeled enzyme horseradish peroxidase (HRP) that the proteins can truly enter the PHEA-*l*-PDMS APCN. The driving force is, most likely, the adsorption of the protein onto the PDMS surface.[29]

Four different enzymes were inserted into the PHEA-*l*-PDMS and their enzymatic activity in the network was compared to that of the native enzyme powder in the respective organic solvent. The activation factor was calculated as the ratio of the activity of the enzyme in the network swollen in the organic solvent divided by that of the enzyme in the same organic solvent but without the network. The results are summarized in Table 1.

As seen from these data, all enzymes are activated upon introduction in the APCNs. HRP and chloroperoxidase are 30 to 110 times more active in the network than in solution.[29] Lipase from *Rhizomucor miehei* is more than 400 times more active when embedded into PHEA-*l*-PDMS[30] than in heptane solution, and even 2000 times when embedded into a hydrophilic/fluorophilic APCN[31] than in supercritical CO_2. α-Chymotrypsin entrapped in PHEA-*l*-PDMS is activated by a factor of 4.500. Addition of salt increases this activation to the 30.000-fold.[32]

Organometal Catalysis

A general problem in catalysis is that one catalyst cannot be used in all media, because of limited solubility and stability. APCNs might be able to overcome this

Table 1.
Enhancement of the catalytic activity of selected enzymes in APCNs. The activation is defined as enzyme activity in the APCN in relation to the activity of the native enzyme powder in the non-aqueous solvent. The numbers in parentheses are the w/w ratios of the two polymer phases in the respective APCN.

Enzyme	APCN system	Solvent	Activation
Horseradish peroxidase	PHEA-*l*-PDMS (77:23)	*n*-heptane	110
Chloroperoxidase	PHEA-*l*-PDMS (77:23)	*n*-heptane	30
α-Chymotrypsin	PHEA-*l*-PDMS (10:70)	*n*-octane	4500
Lipase	PHEA-*l*-PDMS (10:90)	*n*-heptane	440
Lipase	PHEA-*l*-PFPE (30:70)	supercritical CO_2	2000

problem and make organometal catalysts accessible for all media. As an example, we chose a Grubbs–Hoveyda type metathesis catalyst, because there are only a few metathesis reactions known that can be carried out in water. The water-insoluble catalyst was tagged with a perfluoro group to render it fluorophilic.[33] Then the catalyst was loaded into a fluorophilic/hydrophilic conetwork. It could be shown that a loading of up to 10 wt% can be achieved using THF as the solvent.

The loaded APCN was now used to catalyze two different metathesis reactions, shown in Figure 6.

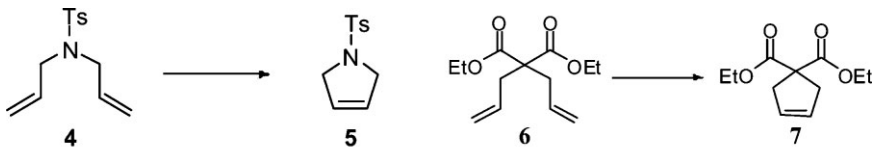
In both cases, using 10 mol% of the catalyst at 60 °C in water, a conversion of 70 to 90% was obtained after 2 h. The conetworks with the higher content of the fluorophilic phase showed higher conversions. However, the second run showed a lower conversion in all cases.

Biosensor

The activation of APCN embedded enzymes in organic solvents combined with the fact that these materials are transparent makes the conetworks interesting matrices for optical biosensors in organic solvents. So far, it was not possible to use biosensors in organic solvents, because the embedded enzymes did not work.

In order to realize such a biosensor, we chose to measure the concentration of organic peroxides, e.g., *t*-butylhydroperoxide, in organic solvents. This is an important field, because the presence of peroxides in organic solvents has been the cause of many accidents and it must, therefore, be controlled frequently.

The basic concept of optical biosensors is the reaction of the substrate with a compound called the chromogen to convert the latter into another compound that gives the optical signal. The optical signal can be a dye or a light emission. In our case, the hydrophilic phase of PHEA-*l*-PDMS conetworks was loaded with the enzyme HRP and 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), a compound that is oxidized to a greenish dye by peroxides in the presence of HRP. According to the concept of phase transfer reactions, the organic peroxide should diffuse into the network via the hydrophobic phase and then be transferred into the hydrophilic phase reacting there with the enzyme and the chromogen. The loading was achieved by simple soaking of the PHEA-*l*-PDMS membranes in an aqueous solution of both compounds. The dried films were then placed into a solution of *t*-butylhydroperoxide in heptane and the increase in absorbance at 402 nm was measured using an UV/vis-Spectrophotometer.[34]



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Substrate	Solvent	APCN Composition	Conversion[a] 1. run	2. run
4	H_2O	30/70	90%	80%
4	H_2O	50/50	60%	40%
6	H_2O	30/70	69%	49%
6	H_2O	50/50	60%	46%

[a] conversion of 0.01 M Substrate at 60°C after 2h (10mol% catalyst).

Figure 6.
Schemes and conversion rates of metathesis reactions in water tested with the perfluoro-tagged Grubbs-Hoveyda catalyst embedded into a PHEA-*l*-PFPE APCN.

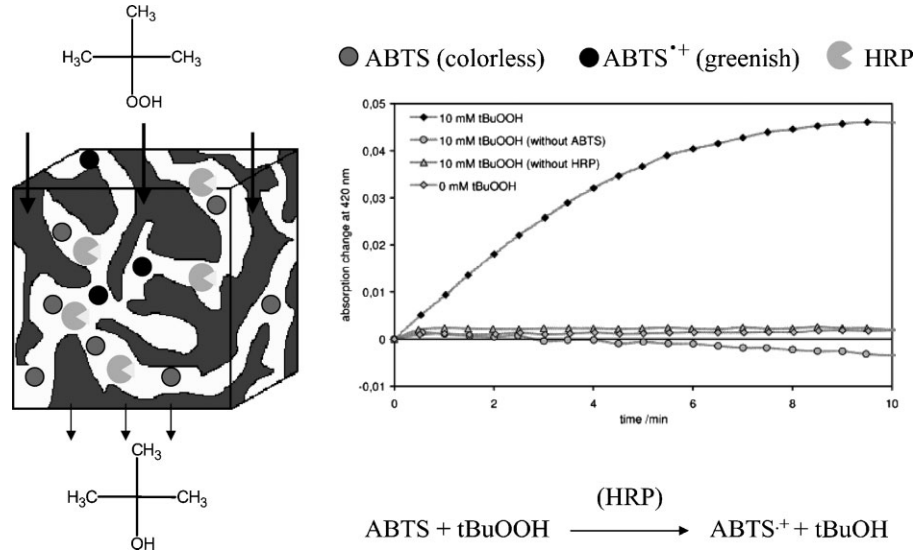

Figure 7.
Concept for a biosensor for peroxides in organic solvents (left) and time dependent increase of absorbance at 420 nm for different scenaria.

Figure 7 depicts the time dependence of the absorbance of the films being unloaded, being loaded with HRP or ABTS only, and being loaded with HRP and ABTS. As is clearly seen from the increase in absorbance, only the film with recognition structure and chromogenic compound does show an increase in absorbance. Thus, the first biosensor in organic solvents could be developed. The implementation of other enzymes for the sensing of other substrates is part of future research.

Gas Sensor

The original application that led to the first APCNs was the soft contact lenses. One of the major requirements in soft contact lenses was high permeability for oxygen. This property was now to be used for the development of an optical gas sensor for oxidizing gases, such as ozone, chlorine, or nitrogen oxides.[24] The perfect optical gas sensor should have the chromogen in a highly gas permeable matrix. Since this is not possible in most cases, the resulting materials lack sensitivity due to diffusion problems. The concept using APCNs for optical gas sensors is again based on the unique property of these materials to combine two different interconnected polymer phases on the nanoscale. Thereby, one phase is highly permeable for gases (e.g. PDMS) and the other shows affinity for the chromogen. Chlorine was chosen as the relevant oxidative gas, because it is present in many environments that are related to the use of bleach. The commonly used chromogen for chlorine is the colorless compound *o*-tolidine. Upon reaction with the gas a violet dye is formed. PHEA-*l*-PDMS conetworks were soaked in an aqueous o-tolidine solution to load the hydrophilic PHEA phase with the chromogen. Two different compositions of the conetworks were used. Sample 1 contained 77 wt% PHEA and 23 wt% PDMS, which should afford a high loading with o-tolidine, but a poor permeability for chlorine. The other sample contained 25 wt% PHEA and 75 wt% PDMS. The conetwork should load less *o*-tolidine, but the chlorine permeability is expected to be excellent, because the PDMS phase is fully interconnected.

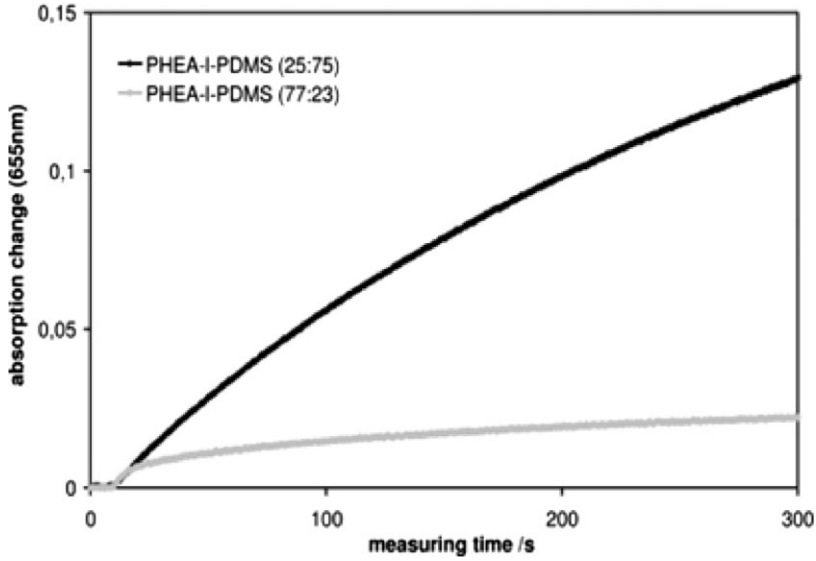

Figure 8.
Absorbance increase of two different *o*-tolidine loaded PHEA-I-PDMS loaded conetworks under chlorine exposure.

Then, the colorless transparent films were exposed to different concentrations of chlorine and the absorbance at 655 nm was measured over time. Figure 8 shows the plot of such an experiment (increase in absorbance over time). It can be seen in this plot that the absorbance increases in both cases. However, the APCN with 75 w% PDMS shows a 4–5 times faster increase of absorbance compared to the higher *o*-tolidine loaded film with 23 wt% PDMS. Expectedly, it could be shown with these data that the major factor in sensitivity of this kind of gas sensor is the permeability of the gas and not the highest amount of loaded chromogen. Compared to an industrial gas sensor design, the APCN-based chlorine sensor is 50 times more sensitive. Thus, APCNs have been found to be the nearly perfect candidates for this application.

In order to demonstrate the versatility of the APCN sensors, the PHEA phase was loaded with a pH indicator and the sensor was subjected to diluted acetic acid gas. Measuring the absorbance of the acidic form of the pH indicator revealed that the conetwork is sensitive for acidic gases as well, although the time for the equilibrium was several minutes. However, the measuring of acetic acid was fully reversible.

Conclusion

APCNs have been shown to act as excellent activating matrices for enzymes in organic solvents and even supports for metathesis reactions in water. Furthermore, the ability to effectively transfer molecules from one phase to another or convert them catalytically at the huge interface opens numerous possibilities for highly effective applications. It could be shown that designs based on APCNs allow biosensing of peroxides in organic solvents for the first time. Gas sensor designs gave highly sensitive matrices for chlorine. Further applications are part of current research.

Besides the phase transfer reactions, APCNs might be highly interesting smart materials. The basis of this application is their ability to switch the percolation of one phase from isolated spheres in a matrix into an interconnected phase.

Acknowledgements: This work was supported by the Deutsche Forschungsgemeinschaft in the SFB 428, and the Emmy-Noether-Program and by the Fonds der Chemischen Industrie.

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