Optimization of the Selectivity of Pyrene Immunoaffinity Columns Prepared by the Sol−Gel Method

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The sol−gel method opened a new path for synthesizing immunoaffinity column packing materials by encapsulating antibodies in the pores of a silica glass matrix. This paper describes different strategies for the optimization of the selectivity of pyrene immunoaffinity columns synthesized by encapsulating the immunoglobulin (IgG) fraction of an anti-pyrene antiserum in a porous silica glass. The selectivity of the columns which are designed for the determination of pyrene in aqueous samples is limited by the retention of cross-reacting and nonspecifically adsorbed polycyclic aromatic hydrocarbons (PAHs). An optimum in selectivity is achieved by a new method to wash the columns with a mixture of a high molecular weight polymer which blocks nonspecific adsorption sites and a surfactant which removes unwanted substances by inclusion into micelles. Optimal results were achieved by washing with 5% (v/v) Roti-Block in 10:90 acetonitrile−water (v/v). Roti-Block is the trade name for a mixture of a high molecular weight poly(vinylpyrrolidone) with a nonionic surfactant dissolved in PBS buffer.

1. Introduction

A large part of analytical chemistry deals with the generation of information on the chemical composition of materials. The data produced are needed to solve problems arising in a wide variety of fields—from material sciences to clinical and environmental chemistry. An ever-increasing number of these problems demands the determination of trace levels of analytes in complex samples. These problems can be solved by applying analytical procedures that consist in separation methods with high selectivity necessary to eliminate interfering matrix components and enrich analyte traces in combination with determination methods allowing the selective determination of the analytes with very low detection limits. Present attempts to solve the problems posed by the determination of nonpolar traces of analytes in aqueous environmental samples mainly use solvent extraction or reversed phase liquid chromatography (solid phase extraction) as separation steps. Both methods have disadvantages: solvent extraction is of limited selectivity and can be used to achieve high enrichment factors only if the bulk of the solvent is evaporated. The evaporation step frequently causes systematic errors due to the loss of more volatile analytes. Although solid-phase extraction offers much better selectivity, the technique approaches its limits when analytes and matrix components are of comparable polarity. One solution of these problems consists in increasing the selectivity by the use of immunoaffinity columns.

Immunoaffinity chromatography uses the highly specific antigen−antibody interactions for the selective separation of antigens from complex mixtures. In this technique antibodies are immobilized in the column packing material. Complementary features of immunoaffinity chromatography and high-performance liquid chromatography (HPLC) with a sensitive detection system allow their off-line or on-line combination resulting in analytical methods of superior selectivity and sensitivity. Immunoaffinity columns have been used in a number of papers for water analysis,[1−5] but columns used were prepared by covalently binding the antibodies to the support material. This immobilization technique frequently leads to changes in the conformation of the antibodies resulting in a loss of affinity for the antigen. The columns used in this paper have been prepared by the sol−gel method which is a mild technique to immobilize biomolecules in the pores of a hydrophilic glass matrix. Compared with other immobilization techniques, the sol−gel method offers a number of advantages.[6−8] Antibodies immobilized by this tech-

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nique largely retain their affinity and specificity to the antigen. The technique avoids activation and bonding reactions needed for covalently binding antibodies to a support material and thus all affinity losses associated with chemical reactions involving the antibodies. The immobilization of antibodies by physical adsorption also proceeds under mild conditions, but it results in columns that are unstable since antibodies are lost during operation by "column bleeding". This effect is absent from immunoaffinity columns packed with materials prepared by the sol−gel method. Since the antibodies are dissolved in the liquid phase contained in the pores, access to their paratopes is not restricted due to the orientation effects caused by most methods for immobilizing antibodies on the support material surface. Immobilization of the antibodies in the pores offers an additional advantage: the exclusion of large molecules by the narrow pore diameter makes such columns ideally suited for the immunoaffinity chromatography of haptens.

The specificity of the columns is, however, compromised not only by cross-reacting compounds but also by nonspecific adsorption on the support material which can be minimized by the selection of a suitable matrix and by appropriate operating conditions. Immobilizing antibodies in a hydrophilic matrix of porous silica reduces the amount of nonspecific adsorption of hydrophobic compounds on the matrix when immunoaffinity chromatography is used for their enrichment from aqueous media. The advantages of the selection of an essentially hydrophilic support material have been demonstrated for the isolation and enrichment of 1-nitropyrene[9] and pyrene[10] from aqueous solutions with affinity columns encapsulating the IgG fraction of antisera against these compounds. The results showed that both analytes were quantitatively retained in the respective columns. In the case of the anti-pyrene columns nonspecific adsorption of EPA−PAHs was absent or very small for compounds with octanol−water partition coefficients log $K_{OW} < 5.2$, but it essentially contributed to the retention of PAHs with log $K_{ow} > 5.2$.

The aim of the present study was to increase the selectivity of a pyrene immunoaffinity column by further reductions in nonspecific adsorption which had to be achieved without compromising the specific retention of the analyte by the antibodies.

2. Experimental Section

2.1. Reagents and Materials. A standard solution containing the 16 EPA−PAHs (naphthalene (NAPH), acenaphthylene, acenaphthene (ACEN), fluorene (FLUO), phenanthrene (PHEN), anthracene (ANTH), fluoranthene (FLUA), pyrene (PYRE), benz[*a*]anthracene (BANT), chrysene (CHRY), benzo[*b*]fluoranthene (BBFL), benzo[*k*]fluoranthene (BKFL), benzo[*a*]pyrene (BPYR), dibenz[*a,h*]anthracene (DIBE), benzo[*ghi*]perylene (BPER), and indeno[1,2,3-*cd*]pyrene (INDE)) was obtained from Supelco (Gland, Switzerland), which also provided chrysene and dibenz[*a,h*]anthracene.

Tween 20 (polyoxyethylenesorbitan monolaurate), Tween 60 (polyoxyethylenesorbitan monostearate), acetonitrile hypergrade, and poly(ethylene glycol) 400 (PEG) were purchased from E. Merck (Darmstadt, Germany). Triton X-405, Triton X-100 hydrogenated, and tetramethoxysilane (TMOS) were obtained from Fluka (Buchs, Switzerland). Roti-Block is the trade name of a blocking agent sold by Karl Roth (Karlsruhe, Germany). It consists of a nonionic detergent and a high molecular weight poly(vinylpyrrolidone) in 10× concentrated PBS.

2.2. Isolation of the IgG Fraction from Polyclonal Rabbit Antiserum. The IgG fraction was isolated from rabbit antiserum by the method of McKinney et al.[11] The amount of IgG calculated by measuring the UV absorption at 280 nm was 4.5 mg of IgG/mL of antiserum.

2.3. Preparation of the Sol−Gel Glass Immunoabsorber.[9] 0.2 mL of 0.04 mol L^{-1} aqueous hydrochloride acid, 0.75 mL of doubly distilled water and 3.4 mL of TMOS were mixed with stirring. The mixture was sonicated under ice-cooling for 30 min.[12,13] After mixing 2 mg of the IgG fraction with 1 mL of PBS, a 1-mL aliquot of the silica sol was added to the ice-cooled antibody solution. Gelation occurred within 2 min, the gel was weighed, and the crystallizing dish was stored at 4 °C for aging. The aging process was stopped when a weight loss of about 50% was achieved. After grinding in a mortar, 0.64 g of the silicate glass (containing 1.3 mg of IgG) was packed into 3 mL glass columns (E. Merck) and washed with 20 mL of PBS. The sol−gel glass immunoabsorber was stored in PBS at 4 C.

Phosphate-buffered saline (PBS, pH 7.6) was prepared by adding 12.46 g of $Na_2HPO_4 \cdot 2\ H_2O$, 1.56 g of $NaH_2PO_4 \cdot 2\ H_2O$, and 8.5 g of NaCl per liter of doubly distilled water.

Composite gels were synthesized by replacing 10% (v/v) or 20% (v/v) of TMOS in the acidic sol reaction mixture by the same volume ratio of PEG 400.

2.4. Operation of Affinity Columns: Loading, Elution, and Regeneration. The immunoaffinity columns were operated with a low-pressure pump (Econo Pump, Model EP-1, BioRad, Hercules, CA) at a flow rate of 1 mL min^{-1}. After preconditioning with 20 mL of 10:90 acetonitrile−water (v/v), the sample solution was applied by pumping 100 mL of a 10:90 acetonitrile−water (v/v) solution containing 0.1 ng of each of the 16 EPA−PAHs through the column resulting in a total load of 160 ng of PAHs. In the following washing step the column was flushed with 20 mL of 10:90 acetonitrile−water (v/v) to remove nonspecifically bound impurities. In some experiments detergents or blocking agents were added to the washing solution. The dissociation of the antigen−antibody complexes resulting in the elution of the trapped compounds was carried out with 40:60 acetonitrile−water (v/v). After elution the columns were regenerated with 20 mL of PBS and stored in PBS at 4 °C.

Breakthrough curves of chrysene were measured by loading the columns with 180 mL of a 10:90 acetonitrile−water (v/v) solution containing 3.5 ng mL^{-1} chrysene.

2.5. Blocking of Potential Adsorption Sites. Adsorption sites on the immunoabsorber surface were blocked with Roti-Block using two different methods. In method 1 the column was blocked by adding 2 mL Roti-Block to 18 mL 10:90 acetonitrile−water (v/v). This solution was used for preconditioning the column before applying the sample solution. In method 2 this solution was used for incubating the column for 1.5 h before preconditioning and application of the sample solution.

2.6. HPLC Determination of PAHs. The chromatographic system used for the determination of PAHs consisted of a high-pressure pump (Model L-6200, E. Merck), a column thermostat (Model bfo-04 dt, W.O. electronics, Langenzersdorf, Austria), a sampling valve equipped with a 20-μL injection loop

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Table 1. Retention Data of PAHs (%) in "Empty" (without IgG) and in Doped Columns Packed with Either TMOS or Composite Glasses

	without IgG			anti-pyrene IgG	
	TMOS	10% PEG	20% PEG	TMOS	10% PEG
naphthalene	n.d.[a]	n.d.	n.d.	n.d.	n.d.
acenaphthene	n.d.	n.d.	n.d.	n.d.	n.d.
fluorene	n.d.	n.d.	n.d.	n.d.	n.d.
phenanthrene	n.d.	n.d.	n.d.	29.9 ± 0.6	26.8 ± 0.6
anthracene	n.d.	n.d.	n.d.	1.2 ± 2.1	1.3 ± 2.1
fluoranthene	n.d.	n.d.	n.d.	84.0 ± 2.9	79.4 ± 2.9
pyrene	n.d.	n.d.	n.d.	101.0 ± 1.4	100.7 ± 1.4
benz[*a*]anthracene	15.3 ± 2.2	5.4 ± 2.3	7.0 ± 2.3	75.6 ± 2.0	70.2 ± 2.0
chrysene	12.6 ± 1.8	1.9 ± 1.9	2.7 ± 1.9	70.0 ± 1.7	63.7 ± 1.7
benzo[*b*]fluoranthene	35.7 ± 3.1	8.3 ± 3.7	14.4 ± 3.5	76.8 ± 2.8	73.7 ± 2.8
benzo[*k*]fluoranthene	38.0 ± 1.9	15.7 ± 2.0	16.3 ± 2.0	68.8 ± 1.8	66.7 ± 1.8
benzo[*a*]pyrene	41.2 ± 2.1	13.6 ± 2.2	13.9 ± 2.2	68.3 ± 2.0	68.8 ± 2.0
dibenz[*a,h*]anthracene	39.0 ± 2.6	31.9 ± 2.6	25.8 ± 2.7	48.5 ± 2.5	47.7 ± 2.5
benzo[*ghi*]perylene	42.1 ± 2.0	23.6 ± 2.2	20.2 ± 2.2	41.4 ± 2.0	46.7 ± 2.0
indeno[1,2,3-*cd*]pyrene	49.0 ± 2.2	29.7 ± 2.4	23.0 ± 2.5	58.6 ± 2.2	57.6 ± 2.2

[a] No retention detected.

(Model 7161, Rheodyne, Cotati), a fluorescence detector (Model F 1080, E. Merck), and an integrator (Model HP 3396 A, Hewlett-Packard, Little Falls, DE). PAHs were separated on a 250 × 4 mm i.d. LiChrospher PAH column (Hewlett-Packard). Gradient elution with an acetonitrile−water gradient and detection by time-programmed fluorescence spectrometry were carried out as described earlier.[10] Acenaphthylene which does not show fluorescence could not be detected. The column was thermostated at 18.0 ± 0.1 °C and operated with a flow rate of 0.6 mL min^{-1}.

In this paper quantitative data are listed with 95% confidence limits based on at least four determinations. Figures include these confidence limits as error bars.

3. Results and Discussion

3.1. Retention Mechanism for PAHs. Substances can be retained in immunoaffinity columns by specific antigen−antibody interactions, by nonspecific interactions with the proteins or by nonspecific adsorption on the immunoabsorber matrix. Experiments with an "empty" matrix not containing antibodies indicate the extent of nonspecific adsorption on the matrix material. Experiments with columns containing nonspecific antibodies will give hints at a possible retention of analytes by nonspecific interactions with the proteins. Information on specific interactions comes from experiments with columns loaded with different amounts of antibodies which exclusively cause differences in specific retention and from comparing the breakthrough curves of a compound measured with an "empty" and an antibody-containing column. In addition, the existence of specific interactions between a compound and an antibody can be shown by ELISA experiments where specific interactions result in cross-reactivity. Cross-reactivity data are, however, imperfect indicators for antigen−antibody interactions in immunoaffinity columns operated far below their saturation since they are obtained in experiments where antigens compete for paratopes on a substoichiometric amount of antibodies. The situation is different in the columns where the antigens meet a large surplus of paratopes. Under these circumstances antigen−antibody interactions of antibodies with broader range of avidity constants will contribute to the specific retention of antigens.

Previous ELISA experiments (unpublished results) with the anti-pyrene antibodies indicated cross-reactivities for benzo[*a*]pyrene (15%), fluoranthene (11%), phenanthrene (10%), benz[*a*]anthracene (1.7%), and benzo[*k*]fluoranthene (1%). The cross-reactivity of the rest of the EPA−PAHs was below 1%. Table 1 presents retention data of PAHs in an immunoaffinity column encapsulating anti-pyrene antibodies (table column 4) and in a column consisting of pure glass matrix (table column 1). Naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene were not adsorbed on the glass matrix. Phenanthrene, fluoranthene, and pyrene are exclusively retained by specific antigen−antibody interactions. The rest of the PAHs is retarded to a varying degree which is the result of both retention mechanisms. Interactions with nonspecific bovine IgG were not observed for any of the substances in earlier experiments.[10] Figure 1 shows the retention data observed for columns encapsulating different amounts of anti-pyrene antibodies. The results indicate the existence of specific interactions for most of the EPA-PAHs with the exception of benzo[*g,h,i*]perylene where the changes observed were statistically insignificant. Figure 2 illustrates the use of break-

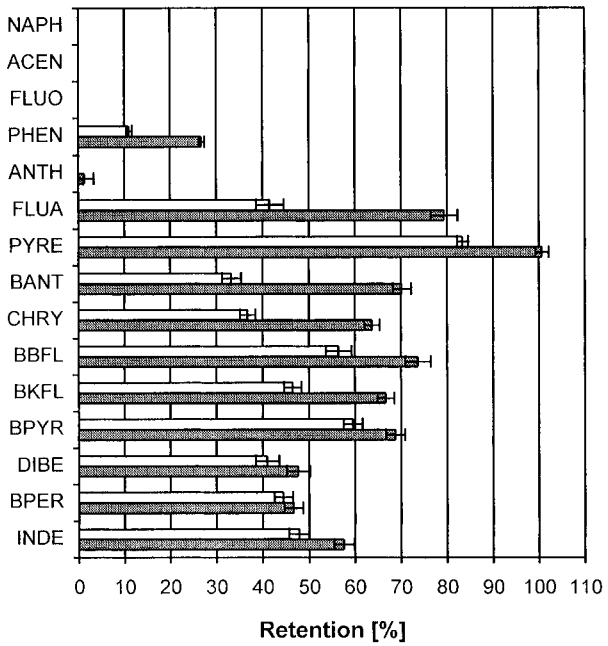

Figure 1. Retention of PAHs in columns loaded with different amounts of antibodies encapsulated in composite glasses (10% PEG). Open bar: column containing 0.9 mg of the IgG fraction of the anti-pyrene antiserum. Gray bar: column containing 1.3 mg of anti-pyrene antibodies.

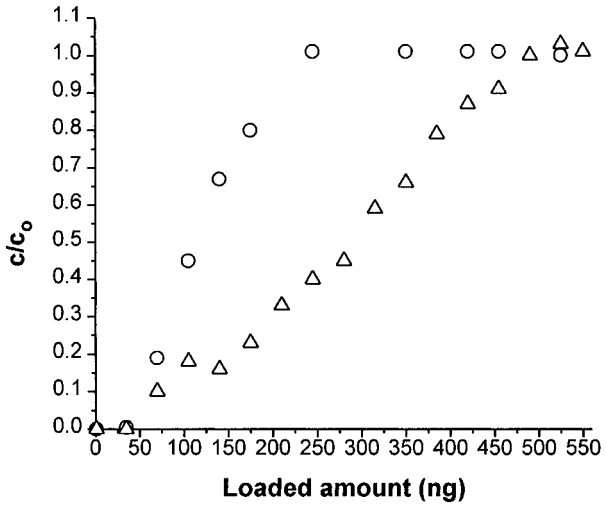

Figure 2. Breakthrough curves of chrysene in columns packed with TMOS glasses. (o) "empty" column (without IgG), (Δ) column with anti-pyrene IgG. Feed solution: 10:90 acetonitrile−water (v/v) containing 3.5 ng mL^{-1} chrysene.

through curves for the example of chrysene which showed a cross-reactivity smaller than 1% in ELISA experiments. The breakthrough curves clearly indicate that the difference is caused by specific interactions with the anti-pyrene antibodies.

3.2. Reduction of Nonspecific Adsorption. In principle a reduction of nonspecific adsorption can be achieved along three different lines: by modifying the matrix to generate a surface with less adsorption sites, by blocking existing adsorption sites with a suitable blocking agent or by adding a detergent to the washing solution to increase the efficiency of washing steps without compromising specific retention by the antibodies.

3.2.1. Modification of the Silica Matrix. Due to the complexity of the polymerization process there is no obvious way to modify the silica matrix which would selectively reduce nonspecific adsorption of PAHs leaving the specific retention properties of the immunoabsorber untouched. Any change in a parameter affecting the hydrolysis rate or the rate of condensation or polymerization steps has an effect beyond changing the surface structure of the silica glass. Synthesizing a silica matrix with properties needed to solve a special analytical problem is—as Avnir[14] put it—"still a matter of art and trial and error". We therefore decided to start from the method we found for synthesizing the anti-pyrene immunoabsorber[10] and tried to modify the structure of the porous silica glass by slightly changing its composition along lines that had proved successful in a similar context.[15] (In this case nonspecific adsorption of atrazine had been prevented by synthesizing composite gels without affecting antigen−antibody interactions).

Sticking to the synthesis procedure already applied successfully for pyrene we synthesized composite gels by replacing part of the precursor TMOS by PEG 400. Table 1 lists retention data obtained with both the TMOS glasses and the composite glasses. Comparing the Table columns 1, 2, and 3 in Table 1 clearly

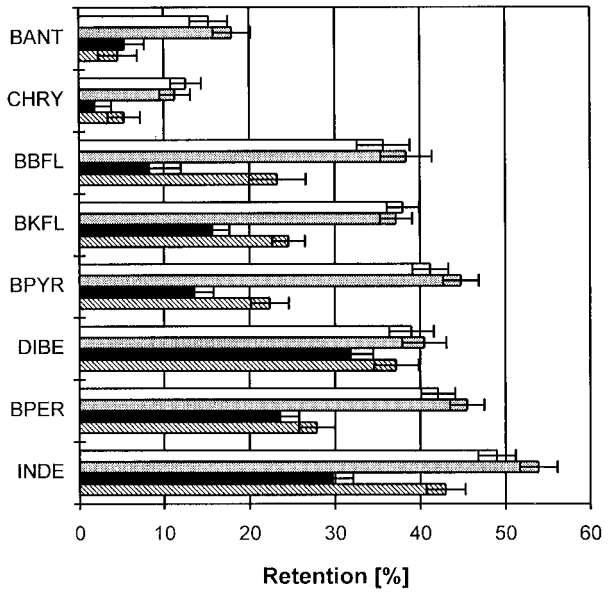

Figure 3. Immobilization of nonspecific antibodies in TMOS and composite glasses: Effect on the retention of PAHs. Open bar: nondoped column packed with TMOS glass. Gray bar: column with bovine antibodies encapsulated in TMOS glass. Solid bar: nondoped column packed with composite glass (10% PEG). Hatched bar: column with bovine antibodies encapsulated in composite glass (10% PEG).

demonstrates that nonspecific adsorption of PAHs is strongly reduced on the "empty" matrix containing 10% PEG. A further reduction of nonspecific adsorption could, however, not be achieved by increasing the amount of PEG to 20% (v/v) since only two compounds showed significant but small differences. The promising results obtained for 10% PEG were largely lost when the IgG fraction of the anti-pyrene antiserum was encapsulated in the composite glass (table column 5). Pyrene was quantitatively retained. Statistically significant reductions were observed for a number of compounds but the effect was small. Figure 3 shows that in contrast to the TMOS glass matrix where the immobilization of nonspecific antibodies did not affect nonspecific adsorption of the EPA−PAHs[10] encapsulating bovine antibodies in the composite glass matrix significantly increased the amount of nonspecific adsorption but was still far below the adsorption rate of the TMOS glass matrix. We can only speculate on an interaction of PEG with protein molecules leading to an increase in nonspecific binding of PAHs without influencing the specific binding properties of anti-pyrene antibodies by inducing conformation changes. Turniansky et al.[15] did not find an increase in the amount of nonspecific adsorption of atrazine using sol−gels doped with IgG from nonimmunized mouse or normal rabbit serum after replacing 10% TMOS by PEG. However, their results showed some differences with regard to the animal species which produced the encapsulated IgG. In addition, they used TMOS/water molar ratios of 1:4 and 1:8 in contrast to the 2.4:1 ratio we used in our experiments; therefore, the concentration of PEG was much higher in our studies. Whether this PEG concentration can effect the antibody structure warrants further investigation. The application of PEG for the chemical precipitation of antibodies is well-known for the separation of antibody bound and free radioactivity in radioimmunoassays.[16]

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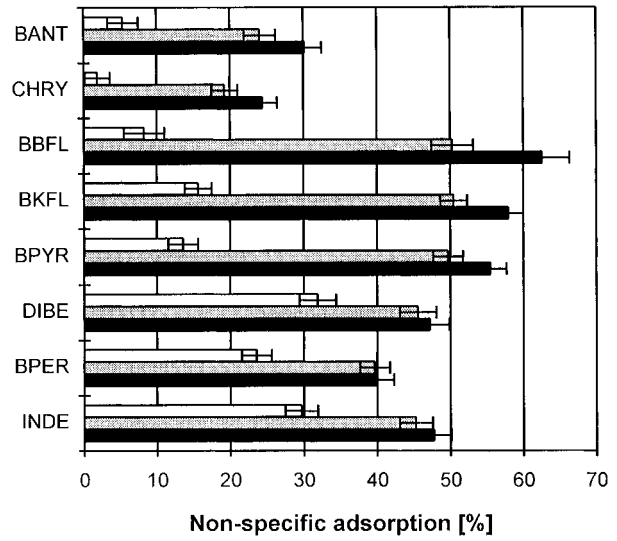
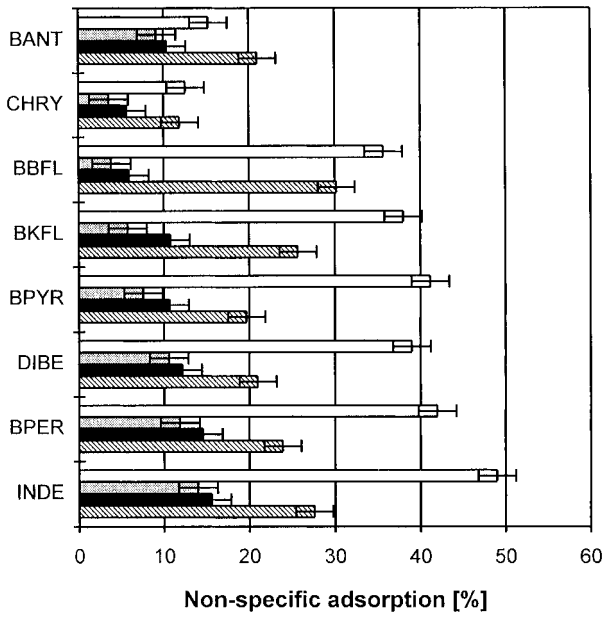


Figure 4. Effect of blocking nonspecific adsorption of PAHs with Roti-Block. Non doped column was packed with composite glass (10% PEG). Open bar: nonblocked column. Gray bar: blocking by method 1 (blocking during preconditioning). Solid bar: blocking by method 2 (blocking before preconditioning).

3.2.2. Blocking of Potential Adsorption Sites. Since we had not been successful in substantially reducing the amount of nonspecific adsorption by modifying the glass matrix we tried to block the adsorption sites of the immunoabsorber surface by applying a blocking agent. The experiments were carried out with nondoped columns packed with composite glass matrix (10% PEG). Figure 4 shows the results of blocking with Roti-Block by two methods. Compared to a nonblocked column both blocking methods dramatically increased the amount of nonspecific adsorption indicating that covering the surface with the blocking agent makes the hydrophilic silica matrix less polar and creates new adsorption sites for PAHs.

3.2.3. Increasing Washing Efficiency by the Use of Surfactants. In immunoassays where nonspecific adsorption to solid surfaces is a well-known problem the addition of detergents to the washing buffer is one of the possible remedies that can decrease the amount of nonspecific adsorption without affecting the specific binding properties of the antibodies. Since nonionic detergents are generally less denaturing to proteins than ionic ones, we restricted our experiments to nonionic detergents which have been added to the washing solution in different concentrations.

In affinity chromatography the efficiency of a washing step using a detergent solution depends on the concentration of a substance in the mobile phase and the degree of its complexation by inclusion in a detergent micelle. For an affinity column in equilibrium the mobile phase concentration of a nonspecifically retained compound is the equilibrium concentration as given by the adsorption equilibrium. In the absence of nonspecific contributions to its retention the mobile phase concentration of a specifically retained compound is determined by the composition of the antibody mixture encapsulated (number of specifically reacting antibody species in the polyclonal antibody mixture, the amount of the different antibody species present and their avidity constants with the analyte). Due to differences in the equilibrium constants involved changing the type and/or concentration of the detergent offers a possibility to optimize a washing procedure: weakly adsorbed analytes or analytes bound to low-affinity antibodies will



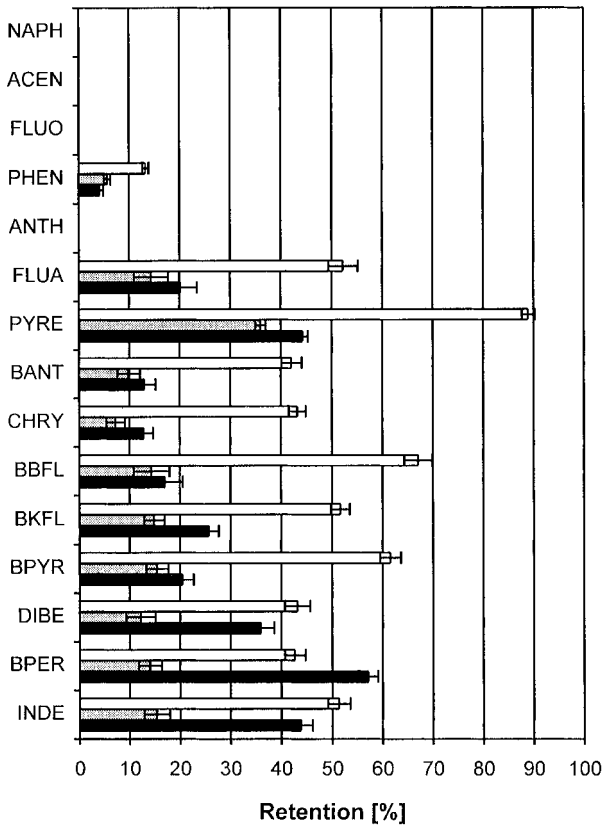


Figure 5. Washing the columns with surfactants: effect on the retention of PAHs. (a, top) Nondoped column packed with TMOS glass. Open bar: washing without a surfactant. Gray bar: washing with 0.5% (v/v) Tween 20. Solid bar: washing with 0.5% (v/v) Tween 60. Hatched bar: washing with 0.5% (v/v) Triton X-100 hydrogenated. (b, bottom) Column with antipyrene antibodies encapsulated in TMOS glass. Open bar: washing without a surfactant. Gray bar: washing with 0.5% (v/v) Tween 20. Solid bar: washing with 0.25% (v/v) Tween 20.

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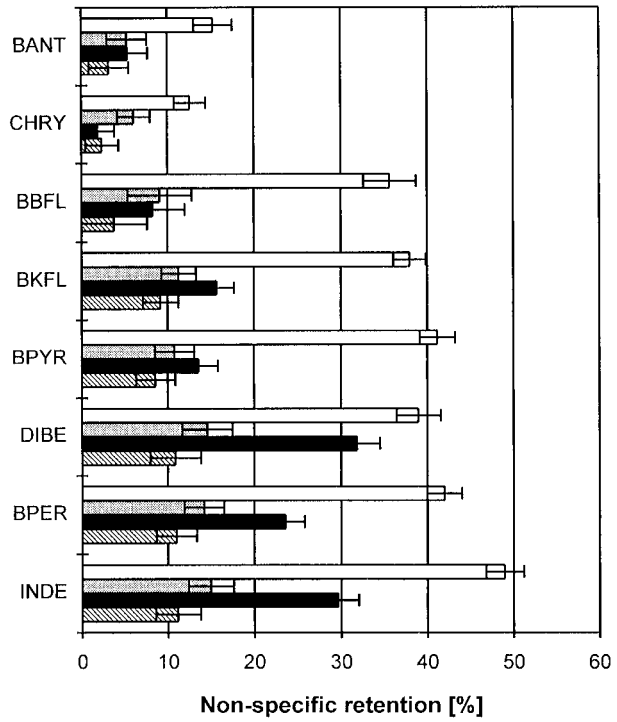

Figure 6. Washing nondoped columns with Roti-Block: effect on nonspecific adsorption of PAHs. Open bar: TMOS glass matrix, washing without Roti-Block. Gray bar: TMOS glass matrix, washing with 5% (v/v) Roti-Block. Solid bar: composite glass (10% PEG), washing without Roti-Block. Hatched bar: composite glass (10% PEG) washing with 5% (v/v) Roti-Block.

dissociate first from the adsorption or antigen binding sites. Increasing the surfactant concentration will increasingly remove the analyte from antibodies with higher affinity constants. Figure 5a shows the results of the removal of PAHs which were nonspecifically adsorbed on a nondoped silica glass matrix by washing with different detergents. The addition of 0.5% (v/v) Tween 20 led to a significant reduction of the amount of PAHs initially adsorbed (reductions: benz[*a*]anthracene 40%, chrysene 72%, benzo[*b*]fluoranthene 89%, benzo[*k*]fluoranthene 85%, benzo[*a*]pyrene 82%, dibenz-[*a,h*]anthracene 73%, benzo[*ghi*]perylene 72%, and indeno[1,2,3-*cd*]pyrene 72%). Only 3−14% of the PAH amounts initially applied were left on the columns. The addition of the same concentration of Tween 60 was slightly less effective. Both detergents were far superior to Triton X-100 hydrogenated. (Triton X-405 could not be used since its fluorescence interferes with the determination of the PAHs.)

As Figure 5b shows these promising results could not be found with analogous experiments with the same matrix encapsulating the IgG fraction of an anti-pyrene antiserum. A surfactant concentration of 0.5% (v/v) was already to high since it strongly interfered with the specific retention of pyrene. This was obviously caused by the removal of pyrene from the dissociation equilibrium of the antigen−antibody complex. (Whether there is an additional effect of the surfactant on the antibody structure leading to a decreased analyte binding remains to be answered. If such interactions exist, they only cause reversible changes, since they disappeared after regenerating the column.) *Repeating* the experiment with 0.25% (v/v) of Tween 20 showed a small increase in the retention of pyrene but the retention of all other interfering PAHs increased as well. A further

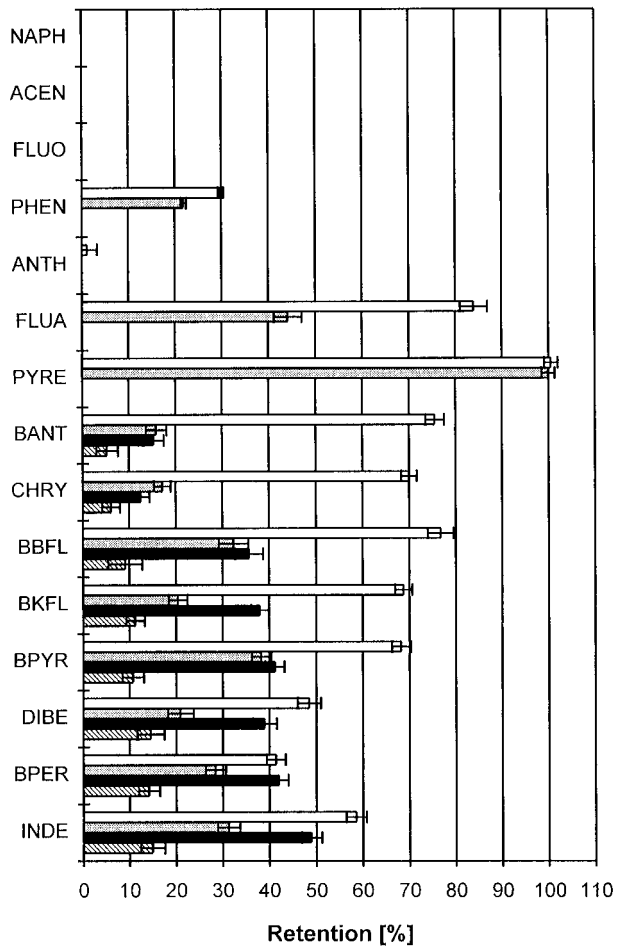

Figure 7. Washing the columns with Roti-Block: effect on the retention of PAHs. Columns packed with TMOS glass. Open bar: anti-pyrene antibodies encapsulated, washing without Roti-Block. Gray bar: anti-pyrene antibodies encapsulated, washing with 5% (v/v) Roti-Block. Solid bar: nondoped column, washing without Roti-Block. Hatched bar: nondoped column, washing with 5% (v/v) Roti-Block.

decrease of the detergent concentration does not promise a significant increase of the selectivity of the anti-pyrene column.

3.2.4. Washing with a Combination of a High Molecular Weight Poly(vinylpyrrolidone) with a Nonionic Surfactant. Since blocking with Roti-Block before the application of the sample increased nonspecific adsorption and effective washing with detergents reduced the specific retention of pyrene, we investigated if washing with Roti-Block would yield better results caused by a combination of two effects: the high molecular weight poly(vinylpyrrolidone) could displace PAHs from the adsorption sites and the nonionic detergent could bind desorbed PAHs by including them into micelles.

Figure 6 shows the results of washing "empty" matrixes with a solution containing 5% (v/v) Roti-Block in 10% (v/v) acetonitrile. Nonspecific adsorption was drastically reduced for the two "empty" matrixes investigated. Significant differences in nonspecific adsorption that had been observed in washing without Roti-Block disappeared. To investigate if this washing procedure interferes with the specific retention of pyrene, the experiment was repeated with an immunoaffinity column encapsulating anti-pyrene antibodies in the TMOS glass matrix. The results obtained are shown in Figure 7. Washing with 5% (v/v) Roti-Block

not only removed nonspecifically adsorbed PAHs to a large extent but obviously also decreased specific retention. This is revealed by the reduced retention of phenanthrene and fluoranthene which—as shown in Table 1—are exclusively retained by specific interactions. Since pyrene was quantitatively recovered in this experiment, washing with 5% (v/v) Roti-Block increased the selectivity of the column for the analyte. An additional increase in selectivity by increasing the Roti-Block concentration to 10% (v/v) could not be achieved since the higher concentration caused a drop in pyrene recovery to 83%. Using 5% (v/v) Roti-Block in the washing solution results in the optimum in selectivity.

4. Conclusion

The results presented here continue our study of the optimization of the preparation and use of sol−gel glass immunoabsorbers entrapping polyclonal anti-pyrene antibodies. Controlling the nonspecific adsorption of structurally related PAHs was attempted along three different lines. Modification of the composition of the glass matrix by copolymerization with PEG 400 only brought minor reductions of nonspecific adsorption. The addition of a nonionic surfactant to the washing solution at concentrations of 0.25% (v/v) or higher significantly removed adsorbed PAHs but unfortunately interfered with the specific retention of the target analyte. The best results were obtained by washing with a mixture of a blocking agent competing with the PAHs for nonspecific adsorption sites and a detergent for including solubilized PAHs into micelles. The addition of 5% (v/v) Roti-Block—a commercially available mixture of a high molecular weight poly(vinylpyrrolidone) with a small concentration of a surfactant—resulted in a column operating procedure offering optimal selectivity for the analyte.

The application of immunoaffinity chromatography for the determination of pyrene served as a model of the problems arising with the selectivity of a sol−gel produced silica matrix encapsulating polyclonal antibodies raised against a nonpolar analyte and used for the determination of the analyte in aqueous solutions. The data hint at the general possibility to optimize the selectivity of antibody-doped sol−gel glasses by the use of different concentrations of more complex washing mixtures such as Roti-Block. If this holds for other antibodies raised against hydrophobic analytes the washing procedure can be adapted for similar problems which will frequently arise with the increased use of sol−gel produced silica matrixes in environmental analysis of aqueous media.

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