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Sol-Gel Entrapment of Monoclonal Anti-Atrazine Antibodies

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Abstract. We report the successful doping of a sol-gel matrix with an antibody, retaining its ability to bind free antigen from an aqueous solution. The particular system described is monoclonal anti-atrazine mouse antibody which was entrapped in SiO_2 sol-gel matrices, prepared from tetramethoxysilane by several methods. Atrazine was selected as a model compound for this study, within the framework of the development of immunochemical-based methods for monitoring pesticide residues and other organo-synthetic environmental contaminants. Nanogram quantities of atrazine were applied on SiO_2 sol-gel columns doped with this antibody, and the amount of eluted antigen was determined by Enzyme Linked ImmunoSorbent Assay (ELISA). Under appropriate sol-gel-forming conditions, high amounts of atrazine were bound to the sol-gels, ranging between 60% and 91% of the amount applied to the column. The combination of the properties of the sol-gel matrix (e.g., stability, inertness, high porosity, high surface area and optical clarity), together with the selectivity and sensitivity of the antibodies, enable extension of this feasibility study to development of a novel group of immunosensors which could be used for purification, concentration and monitoring of a variety of residues from different sources.

Keywords: antibody, atrazine, immunoassay, entrapment, sol-gel, ELISA, pesticide residue, chromatography

1. Introduction

1.1. Entrapment of Proteins in Sol-Gel Matrices

As is evident from the manuscripts in this Special Issue and from several recent reviews [1], entrapment of proteins in sol-gel matrices is one of the fastest growing research directions in sol-gel science and technology. Most of the efforts, so far, have focused on the trapping of enzymes, and the list of successful entrapments of these biocatalysts is growing at a fast pace [1]. Much less attention has been devoted to another large class of reactive proteins, as important as the enzymes, namely, antibodies. It is only lately that preliminary efforts in this direction have begun to appear. We have recently reported preliminary results [2] of a study of the encapsulation of anti-2,4-dinitrobenzene immunoglobulins (IgGs), obtained from a polyclonal antiserum, in SiO_2 sol-gel matrices. That study showed that entrapped IgGs can bind to external antigens (2,4-dinitrophenylhydrazine in that case), which are capable of penetrating the porous network to form the antibody-antigen complex; a full report is in preparation. In another study Livage et al. [3] entrapped the parasitic protozoa cells of *Leishmania donovani infantum* which serve as antigens capable of detecting specific antibodies in serum samples from infected patients. The extent of the antibody-antigen interaction was determined by Enzyme-Linked ImmunoSorbent Assay (ELISA), a commonly used immunochemical quantitative method which is described below. In another

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study Wang et al. [4] encapsulated polyclonal antifluorescein IgGs together with fluorescein in sol-gel matrix prepared from silica sol, demonstrating that the antibody-antigen complex is retained in the matrix, although with a two-orders-of-magnitude-lower affinity constant, compared with solution.

For the convenience of the readers of this Journal, many of whom are unfamiliar with immunochemical methods, we briefly summarize here basic aspects of some immunochemical methods (especially ELISA) which are sensitive tests widely employed as an analytical tool in various formats [5–7] and their importance and implementation in pesticide residue analysis.

1.2. Immunochemical Methods in Pesticide Residue Analysis

Immunochemical methods are playing an increasingly important role in the chemists' and biologists' arsenal of analytical, chromatographic and synthetic techniques, mainly due to their very high sensitivity and specificity [5, 6]. These methods make use of the high binding constants of antibodies to their haptens, affording high sensitivity and—in some cases where antibodies show little cross-reactivity with closely related compounds—also high selectivity. The advantages introduced by the immunochemical methods made immunoassay technology a useful tool in biomedical research as well as in clinical diagnostics. In recent years, some of the immunoassay technologies applied in the biomedical field were transferred to environmental analysis, and concrete evidence for the recent introduction of a number of immunoassays for the analysis of pesticide residues and other environmental contaminants has been presented in the scientific literature [7–9]. Todate, only a limited number of antibodies are available for the analysis of pesticide residues. Since environmental contamination and pesticide residues are routinely monitored worldwide, the need for fast, reliable, on-site and inexpensive analytical procedures is obvious. Currently, monitoring of environmental contaminants and pesticide residues is done mainly by the classical analytical methods which employ gas-liquid chromatography (GLC) in combination with mass spectrometry (MS). These commonly used analytical methods are time-consuming, expensive, require long and complicated purification procedures, and cannot be performed on-site. In addition, some classes of contaminants are difficult to detect by the commonly used GLC-MS methods. As a consequence, much effort has been expended lately into the development of new and improved methods.

Development of a diagnostic immunoassay technology, in general, requires first and foremost an accurate and sensitive quantitative assay. Development of a diagnostic immunoassay technology for detection of pesticide residues and environmental contaminants requires, in addition to the assay itself, the establishment of simple cleanup and concentration procedures, since the analytes have to be monitored in diverse and complex environments (e.g., food extracts, soil extracts, sediments, etc.). One promising approach for cleanup and concentration is the use of immunoaffinity purification, which is then to be followed by a quantitative determination of the analyte. This report concentrates on the first stage of this procedure.

1.3. Immunoaffinity Purification

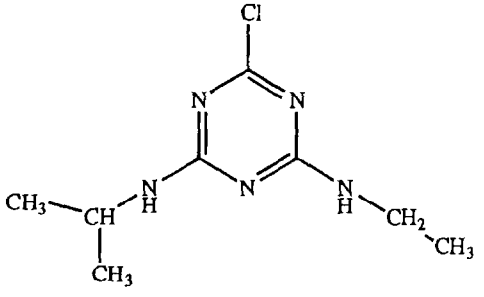
Immunoaffinity purification is a form of chromatography in which antigens or antibodies are immobilized on a solid phase support, and used to bind the required compound selectively. The high binding constants of antibodies to antigens make them an ideal tool for chromatographic separations in a single step. By these immunochromatographic methods, an antibody reared against a specific molecule will, in principle, be able to bind it, with high affinity, from a mixture of compounds. The bound compound can then be released from the antibody by changing the pH of an eluting solution, use of high salt or denaturing reagents, competition with another antigen having a higher affinity for the antibody, etc.

The advantages introduced by immunoaffinity purification made it one of the most powerful techniques for purification of many proteins [10]. These advantages make this method attractive also for application to chemical residue cleanup and concentration from matrices subjected to residue analysis. Although immunoaffinity chromatography has been in use for over a decade, it has not been implemented for the analysis of pesticides and environmental contaminant residues. Successful detection through immunoaffinity purification requires immobilization of the antibodies, and in many cases the immobilization is a lengthy, multi-step process. Consequently, there is a definite need for simplified methods of antibody immobilization and the introduction of new, simple and non-reactive matrices, in order to exploit the full analytical potential of immunoaffinity purification.

We propose a novel method that merges two domains, namely, immunochemical procedures and sol-gel matrices, which carries the potential of providing suitable solutions to these requirements.

1.4. Atrazine/Anti-Atrazine as a Model System

In this study we concentrated on the entrapment of an anti-atrazine antibody in an SiO_2 sol-gel matrix. The selection of atrazine (1) [2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine] as a model analyte is based on the fact that atrazine has been widely used as a herbicide since 1959 [11], and is applied on a global scale of thousands of tons per year [12]. Due to its relatively slow degradation rate [13–16], atrazine residues have been continuously detected in soil, water and groundwater [17, 18].



Atrazine (1)

The antibody we have used is monoclonal, originating from a single cell population of hybrid mouse lymphocytes [19]. Monoclonal antibodies have identical antigen-binding capabilities which improve and simplify experimental procedures greatly, as compared with polyclonal antibodies which are formed by many cells, resulting in a heterogeneous population with varying characteristics.

In this study we report the successful entrapment of an anti-atrazine antibody in a SiO_2 sol-gel matrix, retaining its ability to bind antigen from aqueous solutions.

2. Experimental Details

2.1. Sol-Gel Preparation

Tetramethoxysilane (TMOS, ABCR, 99%) was used in all gel preparations. Methyltrimethoxysilane (MTMOS, ABCR, 99%) and polyethyleneglycol (PEG, Merck analytical; average molecular weight of 400 gr/mole, which corresponds to approximately 7 methylene units in the chain) were also used in some of the gel preparations. The water used was triple-distilled (TDW) (Barnstead NANOpure apparatus). Sonication of samples was carried out in a Branson model R-3, 55 watt, 0.5 liter sonicator. Two methods, namely a two-step method (hydrolysis followed by polymerization [20] (Method I) and direct polymerization of TMOS [21] (Method II), were tested under various conditions for the preparation of the gels and xerogels.

2.1.1. Method I. An acidic silica sol solution was obtained by mixing TMOS with a 2, 4 or 8 molar ratio of 2.5 mM HCl in TDW. The mixture was stirred for one minute until a clear solution was obtained and then sonicated for 30 minutes. The proteins to be encapsulated were premixed either with 30 mM NaCl dissolved in 10 mM sodium phosphate buffer of pH 8.0 (Buffer 1) or HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, Sigmapure 99.99%) buffer (50 mM, pH 7.4). These proteins were either the anti-atrazine monoclonal antibody (AM7B2, kindly provided by Dr. A. Karu, University of California, Berkeley, CA, USA) [22] or non-immunized mouse or rabbit IgG (Sigma, St. Louis, MO, USA). An equivolume amount of the sol was added to the buffer containing the protein to be encapsulated, and the solution was quickly mixed for 5 seconds. Gelation occurred within 1–2 minutes. The reaction was usually carried out in vials immersed in an ice-water mixture. Protein concentrations were 0.6 mg in 1 ml total gel volume (unless otherwise indicated). After 15 minutes the gels were covered with 2–5 ml of buffer and kept wet at 4°C until use. Tests with Merck Neutralit papers, carried out immediately after mixing, showed that the HEPES buffer retains a solution pH of 7.4, while the phosphate buffer's pH decreases to approximately 6.5. Composite gels were made by replacing 10% of the TMOS with PEG (v/v) when preparing the acidic sol.

2.1.2. Method II. 1 ml of TMOS was mixed with 10 ml (an approx. $r = 82 : 1$(!) water: silane molar ratio) of one of the above buffers. The proteins to be encapsulated (anti-atrazine, or non-immunized mouse or rabbit IgG) were premixed into one of the buffer solutions mentioned above. Protein concentration was 0.38 mg in approximately 1 ml of total gel volume. The mixture was stirred for approximately 3 minutes, until a clear sol was obtained. Gelation occurred within another

5–10 minutes. The reaction was usually carried out in vials immersed in an ice-water mixture. The resulting gels were aged for 24 h at 4°C and then lyophilized until a xerogel was obtained as a fine white powder. The doped xerogels were kept at 4°C until use.

A variation of this procedure was performed by exchanging equimolar parts of TMOS with MTMOS to produce a methyl derivatized silicate matrix. Thus, 5%, 10%, 25% and 50% of the total molar amount of TMOS was exchanged with equimolar amounts of MTMOS.

2.2. *Surface Area and Porosity Analysis*

Surface areas and porosities of the protein-doped xerogels were determined from nitrogen adsorption-desorption isotherms, carried out at liquid nitrogen temperature. The data were collected using a Micromeritics ASAP 2000 Surface Analyzer using the generic operating and data analysis software (version 3.01). Nitrogen used was 99.999 pure (Merkaz Hahamtzan). The raw data were analyzed using the BET [23] equation (5 points analysis), and the pore analysis by the BJH [24] model.

2.3. *Atrazine Binding to Sol-Gel*

Wet gels were thoroughly crushed with a spatula, transferred into 5 ml inverted plastic syringes, and packed in 1 ml columns. Dry gels were packed similarly. For optimal activity, columns were kept under buffer at all times during the experiment. Sol-gel columns were washed, prior to sample application, with 50 ml of Buffer 1. Two hundred μl (out of a stock solution of 10 ng/ml, prepared in Buffer 1) of atrazine (analytical grade, 98–99%, kindly provided by Agan Chemicals, Ashdod, Israel) were applied on each sol-gel column (unless otherwise indicated). The eluent was collected and applied once again on the column to allow maximal binding. Columns were washed with 20 ml of Buffer which was collected in fractions of 1 ml. Fractions, containing unbound atrazine, were dried by a Speed Vac (Savant, Farmingdale, NY, USA) and kept at −20°C until use. Samples were resuspended in 200 μl of double-distilled water (DDW), prior to analysis, and tested for atrazine content by the two step competitive ELISA (see below).

The procedure was performed on gels containing the mouse monoclonal antibodies, on non-doped gels or on gels containing non-immunized mouse IgGs (NMS-IgG) or non-immunized rabbit IgG (NRS-IgG). The two latter columns served as the control, and were used to determine the loss of atrazine in the course of the experimental procedure (which may result mainly from non-specific chemisorption). Fractions collected from control and experimental columns were analyzed for their atrazine content, and elution profiles were compared, as shown in Fig. 1.

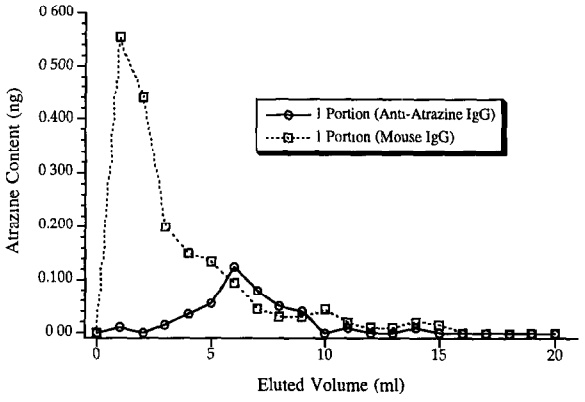
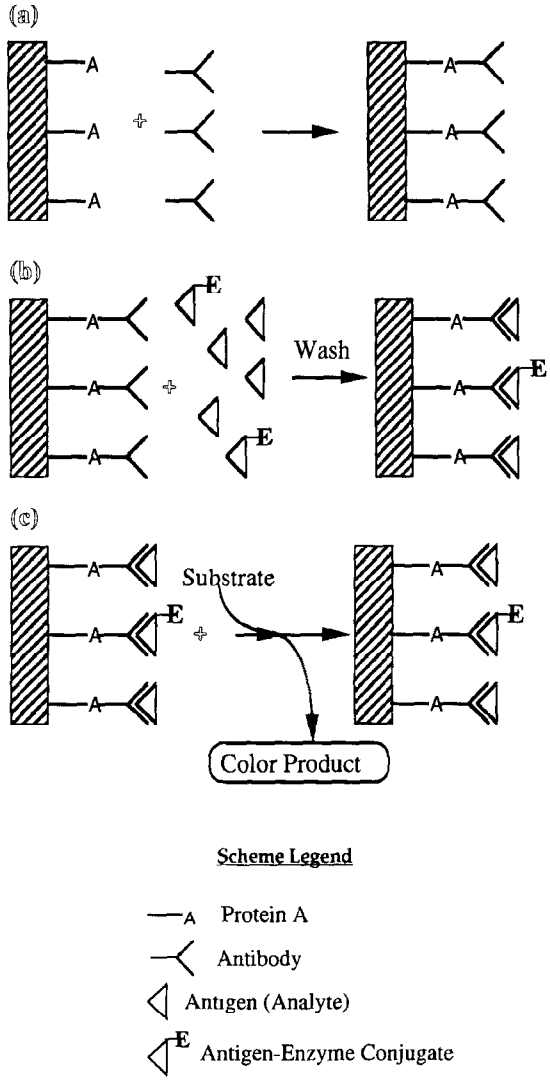


Figure 1. Atrazine elution profiles through anti-atrazine and NMS-IgG doped sol-gels. Gels were prepared by Method I using an r ratio of 1.8 + PEG. One protein equivalent (1 portion defined as 0.6 mg Mab or NMS-IgG) was entrapped in each gel. Two ng of atrazine were applied on each column and the content of atrazine in each fraction was determined by ELISA. Atrazine content in each fraction was calculated from a standard curve. Each fraction was tested, in duplicate, at three dilution (undiluted, 1 : 2 and 1 : 4) which paralleled the atrazine standard curve.

2.4. *ELISA Test*

2.4.1. Background. The assay, in general, couples the high sensitivity and selectivity of antibodies toward their antigens with the signal amplification abilities of a "reporting" enzyme (e.g., alkaline phosphatase or horseradish peroxidase). Thus, a high sensitivity analysis is coupled with the ability to collect a reliable signal, such as that formed by a colorimetric enzymatic reaction. One format of the ELISA test, used in this study, is the two-step competitive ELISA, a test in which both the analyte and an analyte-enzyme conjugate compete for the same binding sites of appropriate antibodies. First, a vessel is coated with antibodies raised against the analyte *via* protein A, which binds to the Fc domain of the antibody ((a) in Scheme 1). Then, a sample containing both the analyte and the analyte-enzyme conjugate are incubated within the vessel, competing for the same limited number of binding sites on the antibodies ((b) in Scheme 1). The vessel



Scheme 1. Scheme of competitive ELISA.

is washed, leaving behind the antigen (the analyte) and the antigen-enzyme conjugate bound to the antibodies, where the analyte/conjugate ratio is determined by the amount of analyte originally present in the sample. A substrate is added, one which is converted by the enzyme (in the conjugate) to a product with a high extinction coefficient, enabling easy quantitative detection ((c) in Scheme 1). The light absorption of the sample is, thus, inversely proportional to the amount of analyte originally present, because the less analyte present—the more enzyme conjugate is left bound and the more product is formed. The enzyme, thus, serves as an amplifier, each enzyme signaling a vacant binding site on an antibody (meaning no analyte is bound to it) with a large quantity of product molecules. With appropriate calibration curves, the test is a powerful quantitative analytical tool. Appropriate measures must be taken to check the cross reactivity of the antibody with other analytes possibly present in the sample.

2.4.2. Two Step Competitive ELISA Test. Two-step competitive ELISA was performed essentially as described previously [25]. Wells of 96-well microtiter plates (NUNC, Maxisorp, Roskilde, Denmark) were coated with 100 μl of 1 mg/1 ml of protein A (Sigma, St. Louis, MO, USA) diluted in 0.5*M* Na_2CO_3 buffer, pH 9.6. After overnight incubation at 4°C, wells were washed with 0.15*M* NaCl in 50 mM sodium phosphate buffer, pH 7.5 (PBS), containing 0.1% v/v Tween-20 (PBST). One hundred μl of anti-atrazine monoclonal antibody, diluted to 1 : 1,000 in 0.5*M* Na_2CO_3 buffer, pH 9.6, was added to the wells, and incubated overnight at 4°C. At the end of the incubation, plates were washed as above with PBST, and 50 μl of each tested fraction or of standard atrazine (ranging from 0.001 to 2 ng/well), in duplicates, was incubated together with 50 μl of horseradish peroxidase (HRP) atrazine tracer (kindly provided by Prof. Bruce Hammock, University of California, Davis, CA, USA) diluted to 1 : 5,000 in PBST. Plates were incubated for 1 h at room temperature, rinsed with PBST as above, and tested for HRP activity using 100 μl of substrate solution which contained 96 μg/ml tetramethylbenzidine (TMB) and 0.004% H_2O_2 in 0.1*M* sodium acetate buffer, pH 5.5. HRP activity was terminated by the addition of 50 μl of 4*M* sulfuric acid. Colour formed by oxidation of TMB in the wells [26] was monitored at 450 nm, using a Labsystems Multiskan ELISA reader. Atrazine content in each of the tested fractions was determined from an atrazine standard curve. In this way elution profiles were obtained and total atrazine binding could be calculated (Fig. 1).

3. Results and Discussion

It has been the accumulated experience of our laboratories as well as of others, that a successful entrapment of dopants requires substantial screening of the sol-gel preparation procedure parameters, and that a fully rational design of an optimal procedure is a goal yet to be achieved. Still, at least partial rationalization is possible, and therefore we share with the reader, in the following section, the route of the search for an optimal procedure.

Table 1. Binding of atrazine to sol-gel columns containing anti-atrazine antibodies or IgG from non-immunized rabbit or mouse.

	Atrazine elution (%)			Atrazine binding (%)	
Sol-gel format	NMS-IgG	NRS-IgG	Atrazine Mab	Total	Net
Dry gels	93	n.t.	87	13	6
Wet gels					
1 : 4	30	44	0	100	30
1 : 4 + 10% PEG	57	61	4	96	53
1 : 8	42	76	0	100	42
1 : 8 + 10% PEG	100	98	9	91	91

Dry gels were prepared by Method II, and wet gels by Method I. 1 : 4 and 1 : 8 signify the TMOS : aqueous HCl molar ratios. Eluted atrazine levels in each sol-gel column were determined by ELISA (see legend to Fig. 1) and are presented as a percent of the total amount of atrazine applied to the column (2 ng). Total binding of atrazine represents the difference (in %) between the total amount of atrazine applied on the column (defined as 100%) and the amount that was eluted from it. Net binding represents the difference between the percent of total binding and the percent of atrazine that could not be recovered using sol-gels doped with IgG from non-immunized mouse serum. Dry gels and wet gels contained 0.38 mg and 0.60 mg, respectively of the protein/1 ml of gel. Mab—monoclonal antibodies; NMS—normal mouse serum; NRS—normal rabbit serum; n.t.—not tested.

We began the gel preparations by trying to minimize possible damage to the anti-atrazine monoclonal antibodies by the methanol formation during hydrolysis and by the drastic changes in pH which occur during gel formation. Thus, we chose Method II with an extremely high r value of 82 as a possible way of diluting the methanol formed during gel preparation and providing a buffered surrounding. The gels formed by this method showed very low binding, and the total amount of atrazine bound to the doped sol-gels represented only 13% of the atrazine applied on the column (Table 1), possibly because the complete drying of the gels damaged the antibodies.

In an attempt to improve binding, we considered examining the binding properties of dry gels with reduced polarity and rigidity of the cage. We prepared such gels by copolymerization with the methyl derivative, MTMOS [27], using the variation of Method II which employs varying concentrations of MTMOS with TMOS. Since atrazine tends to be retained by hydrophobic surfaces, our experiments were first aimed at the preliminary testing of the chromatographic behavior of atrazine on the methylated silica gels in the absence of antibodies. The characteristics of these sol-gel matrices and their non-specific binding properties are summarized in Table 2. The resulting xerogels were mostly

Table 2. Properties of non-doped methylated xerogels.

Molar % of MTMOS	BET surface area (m^2/gr)	BET C value	Average pore diameter (Å) (BJH)*	Isotherm type	Atrazine retained (%)
0%	248	388	28	1	16
5%	544	188	25	1	39
10%	627	119	26	1	29
25%	654	81	27	1	83
50%	496	46	34	2**	100

Gels were prepared by Method II. Amount of retained atrazine represents the difference (in %) between the total amount of atrazine applied on the column (20 ng, defined as 100%) and the amount that was eluted from the gels.

*As determined from the desorption data.

**With hysteresis.

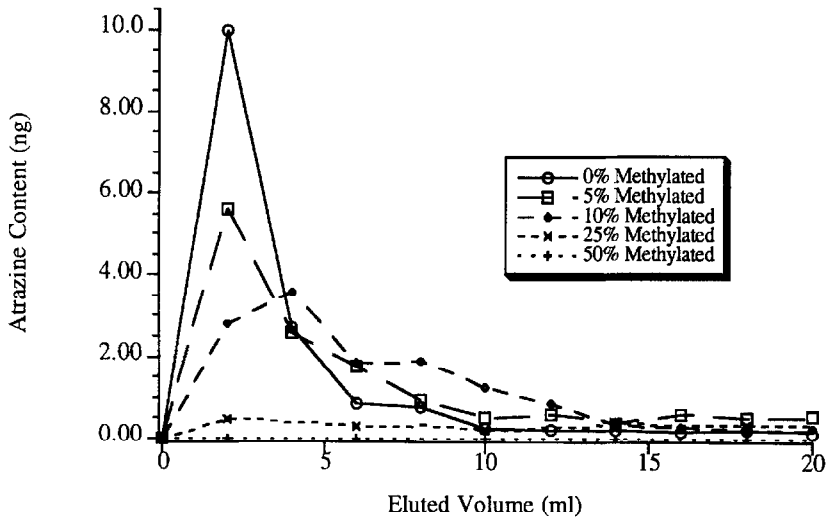


Figure 2. Atrazine elution profiles through non-doped methylated xerogels. All gels were prepared by Method II. Twenty ng atrazine were applied on each column and the content of atrazine in each fraction was determined by ELISA. Atrazine content was calculated from a standard curve. Each fraction was tested, in duplicate, at three dilutions (1 : 4, 1 : 8 and 1 : 16) which paralleled the atrazine standard curve.

microporous with relatively large surface areas. The decreasing value of the BET C coefficient shows how the polarity of the silica surface decreases with increasing percentage of methyl groups. Atrazine elution experiments that were performed on the different xerogels revealed (Table 2 and Fig. 2) that increasing derivatization with methyl groups, which increase the silicate surface's hydrophobicity, increases retention of atrazine to the matrix, hindering possible detection of specific binding of atrazine in the case of encapsulated antibodies. These results show that the preferred silica surface for this specific compound is the hydrophilic one.

Our next improvement was to set about preparing matrices which would be hydrophilic yet flexible and not completely dry. We chose Method I as a possible way of avoiding drastic pH changes, and obtained flexibility by the addition of PEG. Thus, four different combinations of wet gels (containing anti-atrazine antibodies) at two r ratios (1 : 4 and 1 : 8), with and without PEG, were tested for their ability to bind atrazine. At each ratio, 10% of the TMOS in one of the sols was replaced by PEG. The addition of PEG, which is generally "friendly" to proteins creates a composite matrix which is somewhat more flexible than ordinary silicate matrices. Indeed, the addition of PEG proved successful in maintaining the catalytic activity of sol-gel entrapped enzymes [21, 28]. The results (Table 1) show that, unlike in the case of dry gels, anti-atrazine antibodies doped in wet hydrophilic gels retain their ability to bind free atrazine from solution. Comparison of the total binding capacity of the anti-atrazine antibodies in each of the sol-gel composites revealed minor differences between the different formats, and the amounts of atrazine that were bound to all four sol-gels were high, ranging between 91% and 100% of the amount applied on the columns (Table 1).

In order to exclude the possibility that the high binding values resulted from entrapment or non-specific adsorption of atrazine to the doped proteins or silica, and in order to determine the recovery of atrazine, we conducted atrazine elution experiments using non-doped, NMS-IgG or NRS-IgG-doped sol-gels. Recovery of atrazine from these columns (defined as the amount of atrazine eluted from the column) was monitored under the same experimental conditions as those used for sol-gels doped with anti-atrazine antibodies. The results showed different recoveries for the different gel composites. Gels with a 1 : 4 ratio exhibited the lowest recovery values (30–44%) and those with 1 : 8 + PEG exhibited the highest values (98–100%) (Table 1). It is important to note that the amounts eluted from columns doped with NRS-IgG were slightly different from those obtained using NMS-IgG (Table 1). The recovery of atrazine from non-doped 1 : 8 wet gels with PEG was 65%. A much higher recovery (93%) was obtained with a dry gel doped with NMS-IgG (Table 1). It is not clear at present what causes the variability in retention of atrazine on the different sol-gel columns. We believe

Table 3. Binding of atrazine to sol-gel columns containing different amounts of Mab or NMS-IgG.

	Atrazine eluted (%)		Atrazine bound (%)	
Sol-gel content	NMS-IgG	Atrazine Mab	Total	Net
1 equivalent Mab	78	18	82	60
1/2 equivalent Mab	77	46	54	31
1/2 equivalent Mab + 1/2 equivalent NMS-IgG	78	29	71	49

All gels were prepared by Method I with 1 : 8 TMOS : aqueous HCl molar ratios + 10% PEG. One protein equivalent equals 0.6 mg Mab or NMS-IgG. All other details are as described in the legend to Table 1.

that differences in the gel structure (resulting from different pore sizes in gels with different *r* ratios, or from the presence of different proteins, or PEG) affect physical entrapment of atrazine and contribute most to this variability. The contribution of non-specific adsorption to the entrapped proteins plays, most likely, a minor role. Since NMS-IgG resemble most closely the anti-atrazine monoclonal antibodies (which are of mouse origin), we used the extent of loss obtained with NMS-IgG (defined as the difference between the amount of atrazine applied on the column and the amount eluted from it) as a "background" value, and subtracted it from the total binding obtained under each set of experimental conditions. We refer to the total binding minus "background" binding as net binding.

Comparison of the net binding of atrazine to the various sol-gel formats revealed that the best binding activity of the entrapped anti-atrazine antibodies was achieved with composite gels having *r* ratio of 1 : 8 + PEG (Table 1). The gel encapsulated antibodies prepared by this protocol bound 91% of the atrazine applied on these sol-gel columns. Addition of PEG had a significant effect on the net binding in both gel formats, whereas a decrease in gel porosity or in the *r* ratio reduced the ability of the antibody to bind atrazine (Table 1).

Employment of half the anti-atrazine antibody load, in 1 : 8 gels + PEG, caused a drop of the total binding from 82% to 54% (Table 3). The net binding revealed a 2-fold decrease (31% and 60%) for half and one antibody equivalents, respectively. Binding of half of the antibody load in the presence of an equivalent amount of NMS-IgG (which was added to bring the total amount of protein in the sol-gel to that present in sol loaded with one equivalent) resulted in 71% total binding and 49% net binding. These data indicate that a decrease in the amount of antibodies entrapped in the sol-gel results in decreased binding. Since the data are preliminary and the reproducibility of the system is not yet satisfactory, it is impossible to determine the exact correlation between the amount of antibodies entrapped in the sol-gel and the binding capacity. The recoveries obtained in the sol-gels were almost identical (78% and 77%, Table 3), strengthening the assumption of a minor role of the entrapped proteins in the non-specific retention of atrazine.

To compare the binding capacity of the doped anti-atrazine antibodies in the 1 : 8 + PEG gels with their binding capacity in solution, anti-atrazine antibodies (at amounts of protein ranging from 1.3 to 0.6 mg) were incubated overnight with different amounts (20, 16, 8 and 4 ng) of atrazine. The results of this experiment revealed that 0.6 mg of anti-atrazine antibody in solution can bind 20 ng of atrazine. The same amount of antibody entrapped in sol-gel (1 : 8 + PEG) binds, during the short time of loading (~5 min), 1.2–1.8 ng (60%–91% of the 2 ng applied on the column, Tables 1 and 3). Comparison of binding capacities in sol-gel and in solution under various incubation conditions is in progress.

In summary, although the data presented in this study are preliminary and the reproducibility of the procedure is not yet satisfactory the study clearly demonstrates the ability of anti-atrazine antibodies to bind free antigen from solution in a dose-dependent manner.

4. Conclusions

In our study, we have extended the range of sol-gel entrapped biomolecules retaining their activity to include monoclonal antibodies. We have shown that antibodies can be successfully immobilized by entrapment in silica matrices prepared by the sol-gel process, while

retaining their ability to bind antigens diffusing from the outside through the porous matrix. Loss of antigen occurs to some extent, but can be largely eliminated by manipulating the parameters affecting the sol-gel process. Satisfactory reproducibility, as well as optimization of the sol-gel technology are still to be achieved. Nanogram quantities of atrazine were bound to the trapped antibodies and the binding ability, as well as the chromatographic elution profiles, were assayed with the use of ELISA tests. Experimental conditions have been identified to retain the antibody binding activity and to maximize recovery. Chromatographic behavior of several xerogels towards atrazine has been determined. Our preliminary results thus provide the basis for the development of a sol-gel-based immunosensor which has the potential to be used for purification, concentration and monitoring of a variety of antigens.

Acknowledgments

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Note

Zühlke et al described the successful development of an immunoadsorber for 1-nitropyrene [29].

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