JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE 2 (1991) 197–201

Potential interest of optical fibres as immunosensors: study of different antigen coupling methods

P. CLAUDON, M. DONNER, J. F. STOLTZ
INSERM U284, C.O.10, Plateau de Brabois, 54511 Vandoeuvre les Nancy, France

New approaches in the field of fluoroimmunoassays involve the use of optical fibres as solid support of reagents and waveguide. Parameters which influence the response of optical fibre fluoroimmunosensors have been studied. Several methods, involving either a physical or a covalent process, have been investigated for immobilizing on silica surfaces polyclonal rabbit immunoglobulin (IgG) as antigen. Fluorescent anti-rabbit IgG has been used for determining immobilized antigen levels. The fluorescence intensity emitted by the immunosensors has been determined by using the evanescent wave phenomenon. The binding capacity of the different immunosensors tested appears nearly similar, except for the sensor prepared with the 3-glycidoxypropyl trimethoxysilane coupling method which seems to exhibit a lower binding capacity. For all sensors prepared with a covalent coupling method, a simultaneous adsorption phenomenon probably affects the long-term stability of immunosensors. In a further step, the possibility of immunosensor regeneration after a dissociation of antigen/antibody complexes has been tested. It appears that the dissociation methods could affect the sensor response. Finally, the specificity of immunosensors has been investigated. A significant cross reactivity was observed for *p*-toluenesulphonyl chloride and 3-aminopropyl triethoxysilane (APTES)-derivatized fibres. For the APTES-derivatized sensor, the specificity markedly decreased after a dissociation step. Therefore, although the feasibility of a competitive assay has been established, suitable conditions of immunosensor regeneration still require further investigation.

1. Introduction

In the last few years, optical methods such as fluorimetry have been increasingly used in the field of immunoassays, because of the lack of drawbacks linked to radioimmunological methods: health hazards, lifetime of kits limited by the half-life of isotopes [1–3]. However, the use of fluorescent probes to replace isotopic labels is hindered by the decreased sensitivity obtained with fluorescence which is caused by the sample's own fluorescence. For these reasons, solid-phase immunoassays have been developed in an attempt to improve the sensitivity of fluorescence methods. In this way, recent efforts have been concentrated on the potential interest of immunosensors based on optical fibres used as both solid phase for antigen or antibody immobilization and light tranducer [4–7]. These immunosensors can be classified into three categories according to the associated instrumentation.

1. Exciting light is focused into the core and guided to the sample. The fluorescence signal returns through the same fibre [8, 9].

2. Optical fibres can be used as fluorescence collector. In that case, the fluorescent molecules at the fibre interface are excited with a classical optical system.

3. Other systems are composed of an optical fibre which is only used to conduct the exciting beam. The optical arrangement for fluorescence detection may be a commercially available system (spectrofluorometer).

Optical fibre sensors offer several advantages [5, 10]. The sensor itself is the solid phase of antigen or antibody immobilization. A silica or quartz fibre is resistant to chemical or heat treatment. The signal attenuation in the waveguide is tiny and it is not subject to electrical and electromagnetic interferences. Furthermore, optical fibres allow the development of miniature sensors using small amounts of biological fluids and reagents. Finally, it is possible to take advantage of an optical phenomenon occurring at the fibre interface. Indeed, although the measurements are usually performed at the distal end of the waveguide, evanescent wave spectrometry is increasingly used in order to monitor the formation of antibody–antigen complexes occurring at interface [9, 11, 12].

Nevertheless, major problems must be solved before considering the use of such immunosensors in routine assays. A satisfactory reproducibility of the results at first requires a complete control of the attachment of antigen or antibody on silica or quartz fibres. Second, the immunosensor must present a long-term stability

0022–2461/91 $03.00 + .12

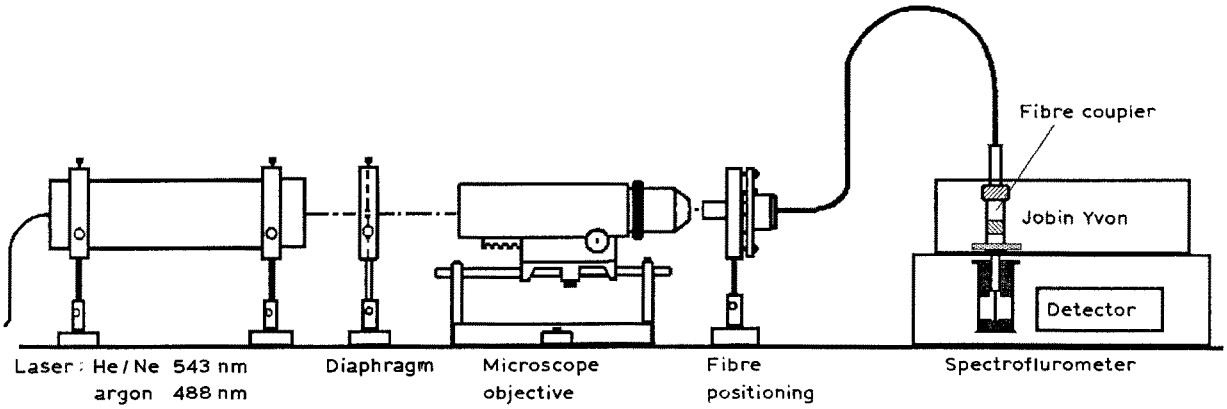

Figure 1 Schematic diagram of the optical device and measuring system.

with a minimal desorption of immobilized biomolecules. Finally, the regeneration of immunosensors after the dissociation of antigen–antibody complexes is still poorly understood.

The present preliminary work represents an approach of these problems by using rabbit immunoglobulin (IgG)/anti-rabbit IgG as a model of antigen/antibody reaction in a competitive assay.

The immobilization of the antigen (rabbit IgG) on the surface of silica fibres has been carried out with six different methods. A covalent attachment has been considered with four techniques: two simple methods involve one single step using either *p*-toluenesulphonyl chloride (TSC) or *p*-nitrophenyl chloroformate (NPCF) as coupling agent. Two methods require the use of an intermediate silane coupling agent: 3-glycidoxypropyl trimethoxysilane (GOPS) or 3-aminopropyl triethoxysilane (APTES). These covalent binding methods have been compared with two techniques involving an adsorption step: a method with a simple adsorption of IgG on a fibre optic surface and another one using adsorption of an amino-carrying polymer [13] (poly-*L*-lysine) followed by the covalent binding of amino groups of IgG and poly-*L*-lysine with glutaraldehyde.

2. Experimental procedure

2.1. Instrumentation

Fig. 1 shows a schematic representation of the optical and detection design. This system includes either a He/Ne laser (543 nm, Melles Griot, Montigny le Bretonneux, France) or an argon laser (488 nm, 2025 Model, Spectra Physics, Mountain View, CA, USA) as exciting source.

The light beam is focused on an optical fibre which guides the light to the immunosensor through the fibre to fibre coupler. This immunosensor is a 10.5 cm length silica multimode optical fibre (core diameter 1 mm, silicone as optical cladding: PCS 1000 W, Quartz and Silice, Paris, France). This fibre is immobilized in a glass cuvette inside a Jobin Yvon JY3D spectrofluorometer (Longjumeau, France) whose detection system is used to determine the fluorescence intensity levels.

2.2. Immunosensor preparation

Before the chemical treatment, the fibres were finely hand polished with lapping film and stripped of 18 mm of jacket and optical cladding. The cladding chemical stripping solution was O.F. Stripper "S" (Lumer, Bagnolet, France). The fibres were cleaned for 4 h in 12.5% HNO_3 and rinsed in water. The rabbit immunoglobulin G coupling procedures were similar to those used by Tromberg *et al.* [8] for GOPS and Williamson *et al.* [14] for APTES, TSC and NPCF.

For the coupling methods involving an adsorption procedure, the first technique was a simple adsorption of rabbit IgG on silica fibres: 0.1 mg ml^{-1} in PBS pH 7.4 over 24 h at 4 °C with gentle stirring. The second one includes a poly-*L*-lysine adsorption on silica fibres. Poly-*L*-lysine was dissolved in doubly-distilled water at the concentration of 0.01% and the sensors incubated for 3 h at 20 °C with gentle stirring. These fibres were dried overnight at 20 °C or 1 h at 55 °C. Then, the glutaraldehyde activation and rabbit IgG coupling method were similar to those used for APTES [14]. For all methods, the rabbit IgG coupling procedure was the same: 0.1 mg ml^{-1} over 24 h at 4 °C with gentle stirring. After IgG coupling, the immunosensors were placed in PBS pH 7.4 with 1% BSA and 0.02% sodium azide at 4 °C. This solution allows both to block the free sites available on silica surface and to conserve immunosensors.

2.3. Antigen–antibody reaction

The immunosensors were incubated with tetramethylrhodamine isothiocyanate (TRITC) or fluorescein isothiocyanate (FITC) labelled polyclonal anti-rabbit IgG (0.02 mg ml^{-1} for 3 h at 37 °C). After incubation, the immunosensors were washed in PBS and the fluorescence intensity was determined. A reference immunosensor, not incubated with labelled anti-rabbit IgG, was used to determine background levels.

3. Results and discussion

The efficiency of various immobilization methods was tested by studying the binding of labelled anti-rabbit IgG on optical fibres prepared by incubation in a

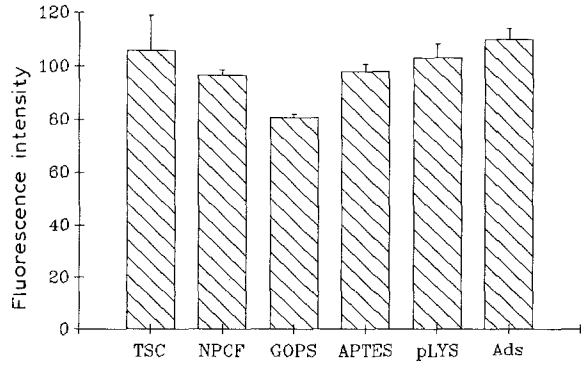

Figure 2 Effect of coupling method on the interaction of rabbit IgG immobilized on silica fibres with anti-rabbit IgG.

rabbit IgG solution (0.1 mg ml^{-1}). The results are summarized in Fig. 2. It appears that the binding capacity of different immunosensors is very similar, except for the sensors prepared with GOPS-derivatized fibres which seem to exhibit a lower binding capacity. It is also observed that the immobilization methods involving adsorption phenomena induce a binding capacity similar to that obtained with covalent coupling methods. These slight differences could be explained by hypotheses suggested by Lin *et al.* [15]. According to these authors, the formation of chemical bonds during the covalent coupling process is probably preceded by an adsorption phenomenon. The adsorption properties should be determined by the nature of both surface and the biomolecules and therefore influence the immobilization process. In our model, it is likely that the observed differences are probably related to the complex silica surface/coupling agent which could exhibit different adsorption properties.

The feasibility of a competitive assay using these immunosensors was studied by adding increasing concentrations of unlabelled anti-rabbit IgG. Fig. 3 shows a typical result with APTES-derivatized immunosensors. Samples containing greater amounts of unlabelled antibodies exhibit lower fluorescence signals. These results indicate that more labelled antibody was displaced from binding sites on fibres and show the feasibility of a competitive assay involving the immunosensors and the optical device.

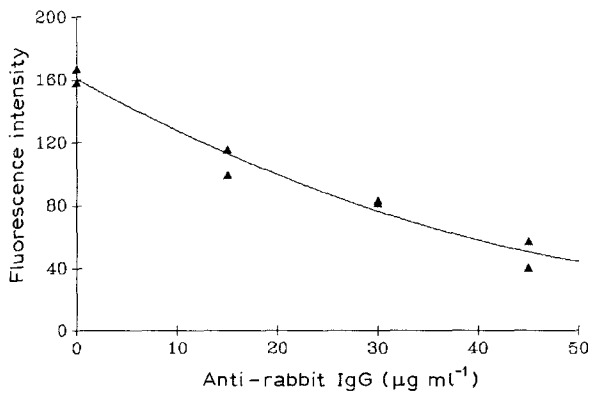

Figure 3 Standard curve of a competitive assay for an anti-rabbit IgG. Immunosensors were prepared with APTES reagent. Relative fluorescence intensity of rabbit IgG/TRITC anti-rabbit IgG is plotted as a function of concentrations of unlabelled anti-rabbit IgG.

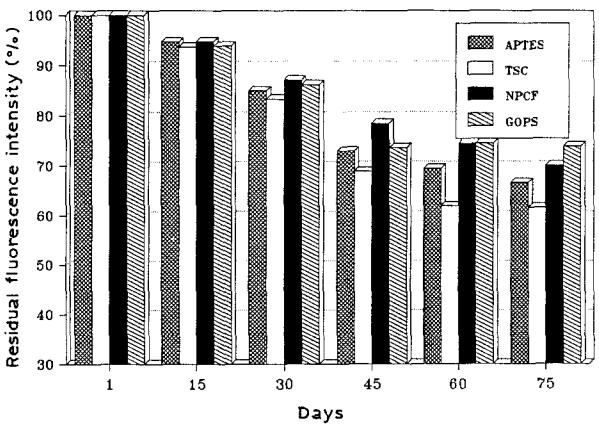

Figure 4 Effect of covalent coupling method on the stability of immunosensors at 4 °C. The relative fluorescence intensity determined immediately after immunosensor preparation was considered to be 100%. The residual intensity expressed in per cent is plotted as a function of storage period of fibres.

In the following step, the stability of immunosensors was examined for approximately 80 days. When stored at 4 °C, the immunosensors prepared with a covalent coupling method lost about 30% of their binding capacity. After a storage period of 45 days, the binding capacity of the immunosensors appeared to be nearly stable (Fig. 4). It might be suggested that this decrease in the binding capacity of anti-rabbit IgG is due to the fraction of rabbit IgG molecules immobilized on fibres via an adsorption phenomenon. To verify the validity of this hypothesis, the preparation of immunosensors was immediately followed by a treatment with 1% sodium dodecyl sulphate (SDS) which is known to release adsorbed biomolecules [12]. Following SDS treatment, it has been found that the initial sensor response decreased by about 20–30% according to the coupling method and that the determined fluorescence levels remained stable for several weeks (data not shown). Therefore, these results are in agreement with the existence of covalently fixed and adsorbed rabbit IgG molecules on optical fibres.

Different methods were then compared for their effect on the dissociation of antigen–antibody complexes and the recovery of immunosensor activity [8, 15–19]. Fig. 5 illustrates the results observed with APTES-derivatized immunosensors. It appears that in our model ethylene glycol, glycine/HCl buffer and 3M KSCN failed to release an important amount of anti-rabbit IgG. In contrast, both phosphoric and propionic acids result in the dissociation of most of the labelled antibodies. As shown in Fig. 5, these immunosensors retained all of their initial activity in a second series of measurements. However, a further dissociation step fails to disrupt the same amount of antigen–antibody complexes. These results could be explained by the acid pH of the dissociating solutions. Lin *et al.* [15] reported that a decrease in the pH induces modifications in antibody structure resulting in an increase in their adsorption properties. In our model we can suggest that acid media could, in the course of dissociation processes, irreversibly bind a fraction of labelled anti-rabbit IgG on the silica surface. With 10% SDS + urea as a dissociating reagent,

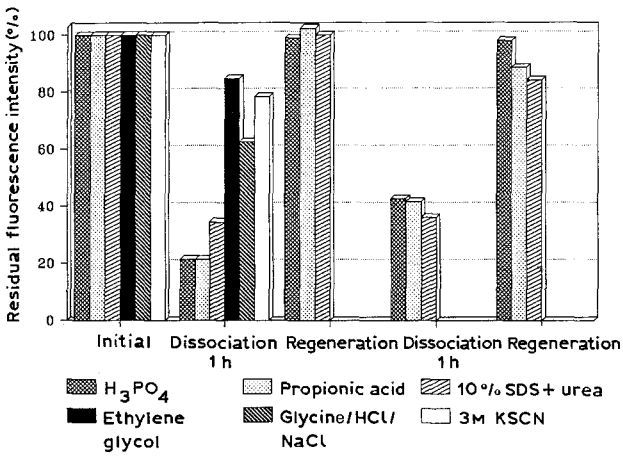

Figure 5 Dissociation of anti-rabbit IgG from immobilized rabbit IgG. Before dissociation procedure, the immunosensors were washed with 1% SDS. For dissociation, the immunosensors were incubated for 1 h at room temperature with a gentle agitation in a dissociating solution (0.1 M H_3PO_4 pH 1.8; 10% propionic acid pH 2.2; 10% SDS + 6 M urea pH 7.5; 50% ethylene glycol pH 3.6; 0.01 M glycine/HCl/0.5 M NaCl pH 2.3; 3 M KSCN pH 5). After the dissociation, the immunosensor is stored in phosphate. Buffered saline (PBS)– = 1% Bovine Serum Albutin (BSA) overnight at 4 °C.

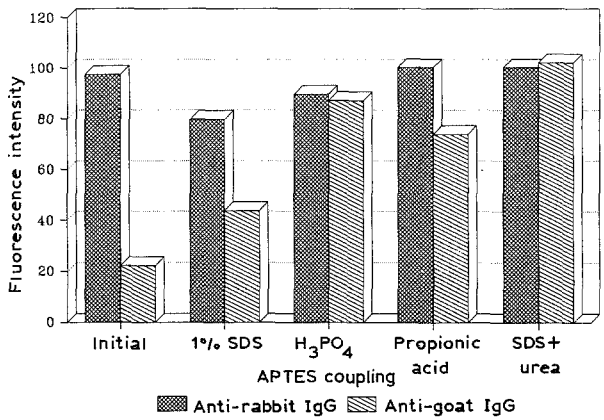

Figure 7 Effect of the dissociation procedure on the specificity of immunosensors prepared with APTES as coupling reagent. Dissociating procedure: H_3PO_4, propionic acid, 10% SDS + 6 M urea. The results were compared to the initial response of untreated immunosensors or only treated with 1% SDS. Fluorescence intensity levels were recorded following the interaction of rabbit IgG with labelled anti-rabbit IgG or anti-goat IgG.

the residual fluorescence signal does not increase after the second dissociation step. Nevertheless, the amount of released antibody appears to be lower than that obtained with acid media. Furthermore, the binding capacity is reduced after two dissociation steps.

The specificity of immunosensors was then determined by performing cross-reactivity experiments with anti-goat IgG. The results are shown in Fig. 6. The binding of anti-goat IgG is very weak for immunosensors derivatized with NPCF, GOPS, treated with poly-*L*-lysine or coated with adsorbed rabbit IgG. In contrast, a significant cross reactivity was observed for TSC- and APTES-derivatized fibres. This lack of specificity, which has also been reported by Lin *et al.* [20] with APTES as coupling agent, is not well understood. It might be suggested that localized conformation changes occur in the course of the rabbit IgG immobilization process and result in a loss of specificity.

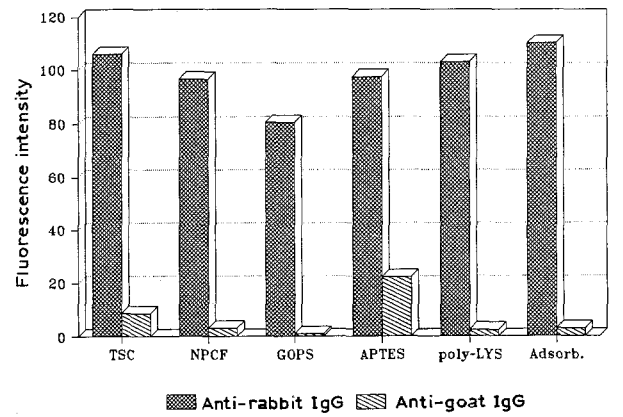

Figure 6 Effect of immobilization method on the specificity of immunosensors. The fluorescence intensity was determined after interaction of immobilized rabbit IgG with labelled anti-rabbit IgG or anti-goat IgG.

The cross reactivity observed with APTES-derivatized sensors markedly increased in the case of a second response after a dissociation step with phosphoric acid, propionic acid and 10% SDS + urea (Fig. 7). It should be noted that the specificity also decreased when the immunosensors are treated with 1% SDS which releases the adsorbed rabbit IgG molecules. The origin of this weak specificity can also be related to the used experimental model. The antigenic sites of rabbit IgG could be altered by dissociating treatments. Further investigations are required to determine whether the various treatments induce similar alterations in the specificity of immobilized antigens.

4. Conclusion

The use of an evanescent wave phenomenon coupled to the immobilization of biomolecules on the surface of optical fibres could represent an improvement in the field of immunosensors. However, it is necessary to optimize parameters which could have a role on detection limits and specificity.

The first parameter which has to be controlled is the immobilization procedure. Our study revealed that the use of a covalent coupling method does not exclude the possibility of a simultaneous adsorption phenomenon which affects the long-term stability of immunosensors. Moreover, the problem raised by immunosensor regeneration requires further investigations. Mild regeneration conditions must be found to retain the specificity of immunosensors.

Acknowledgements

The optical part of the measuring system was developed by J. Didelon and P. Nicolazzi whose cooperation is deeply appreciated. This work was supported in part by Region Lorraine grants DBCR 201-89 and 187-90.

References

1. A. ZHIRI, Biofutur, in "Supplement: Le Technoscope" **6** (1986) 3.
2. B. J. GOULD and V. MARKS, in "Nonisotopic Immunoassay", edited by T. T. Ngo (Plenum Press, New York, London, 1988) p. 3.
3. J. P. GOSLING, *Clin. Chem.* **36** (1990) 1408.
4. W. R. SEITZ, *J. Clin. Lab. Anal.* **1** (1987) 313.
5. W. R. SEITZ, *Anal. Chem.* **56** (1984) 16.
6. R. E. DESSY, *ibid.* **61** (1989) 1079.
7. G. BOISDE and J.-J. PEREZ, *C.R.A.S., Série Générale* **5** (1989) 303.
8. B. J. TROMBERG, M. J. SEPANIAK, T. VO-DINH and G. D. GRIFFIN, *Anal. Chem.* **59** (1987) 1226.
9. J. T. IVES, J. N. LIN and J. D. ANDRADE, *Amer. Biotech. Lab.* **3** (1989) 10.
10. M. S. ABDEL-LATIF, A. SULEIMAN, G. G. GUILBAULT, B. B. A. DREMEL and R. D. SCHMID, *Anal. Lett.* **23** (1990) 375.
11. J. D. ANDRADE, R. A. VANWAGENEN, D. E. GREGONIS, K. NEWBY and J.-N. LIN, *IEEE Trans. Electron Devices* **32** (1985) 1175.
12. K. R. ROGERS, J. J. VALDES and M. E. ELDEFRAWI, *Anal. Biochem.* **182** (1989) 353.
13. K. PACHMANN and W. LEIBOLD, *J. Immunol. Methods* **12** (1976) 81.
14. M. L. WILLIAMSON, D. H. ATHA, D. J. REEDER and P. V. SUNDARAM, *Anal. Lett.* **22** (1989) 803.
15. J. N. LIN, J. D. ANDRADE and I.-N. CHANG, *J. Immunol. Methods* **125** (1989) 67.
16. F. V. BRIGHT, T. A. BETTS and K. S. LITWILER, *Anal. Chem.* **62** (1990) 1065.
17. G. C. BLANCHARD, C. G. TAYLOR, B. R. BUSEY and M. L. WILLIAMSON, *J. Immunol. Methods* **130** (1990) 263.
18. J. L. BOITIEUX, M.-P. BIRON, G. DESMET and D. THOMAS, *Clin. Chem.* **6** (1989) 1026.
19. L. LOCASCIO-BROWN, A. L. PLANT, V. HORVATH and R. A. DURST, *Anal. Chem.* **62** (1990) 2587.
20. J. N. LIN, J. HERRON, J. D. ANDRADE and M. BRIZGYS, *IEEE Trans. Biomed. Eng.* **35** (1988) 466.

Received 8 January
and accepted 8 April 1991