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

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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 transducer [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.
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-toluene sulphonyl 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% HNO₃ 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