A DISPOSABLE OPTICAL BIOSENSOR BASED ON TOTAL INTERNAL REFLECTION FLUORESCENCE

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SUMMARY

A novel disposable biosensor device is described, based on the generation of evanescent light waves at an optical interface. The sensor is injection moulded from high optical quality plastic and consists of two parts, the waveguide and a cuvette for containing the sample solution. Antigens are attached to the waveguide surface and the reaction with antibodies is monitored by exciting and collecting fluorescent light "back-tunneled" out of the waveguide. A model assay for human IgG is used to demonstrate that this biosensor can give rapid, sensitive results.

1 INTRODUCTION

One of the most promising approaches to biosensor transduction mechanism are the optical devices, due to the broad knowledge base both in optical component design and selection, and the concomitant knowledge in the chemical/solution assay systems. We have recently reviewed the major types of such "interface immunoassay" system (1, 2, 3) but will concentrate on describing one concept of such an optical sensor device based on internal reflection spectroscopy, particularly total internal reflection fluorescence (TIRF).

2 TIRF

When a light beam is totally internally-reflected within the optically denser medium, an electromagnetic waveform called the evanescent wave is generated in the rarer medium close to the reflection surface. This evanescent ("disappearing") wave is part of the internally-reflected light beam and penetrates a fraction of a light wavelength into the lower refractive index medium. The evanescent wave can optically interact with compounds distant from the waveguide surface. This optical interaction can be followed in a number of ways of which we will concentrate on Total Internal Reflection Fluorescence (TIRF). In TIRF the adsorption of evanescent photons by surface bound molecules is the first of a two-step process where, in a second step, the photons are re-emitted at a longer wavelength as fluorescence.

We have opted to measure fluorescent light from surface bound molecules which is "tunneled" back into, and exits from, the waveguide in a similar fashion to the excitation light.

3 WAVEGUIDE DESIGN AND RESULTS

The design we selected is shown in Fig. 1. The reagent is affixed onto the interior surface of the waveguide and sample applied via a hole in a cover. Excitation light is guided into the waveguide using a

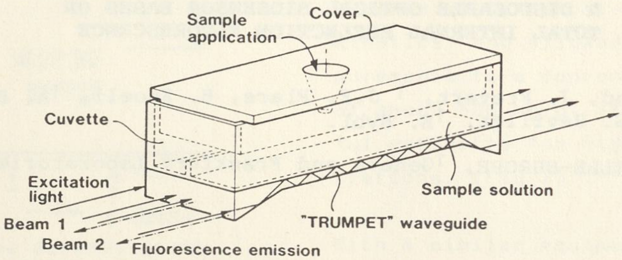

FIGURE 1

wedge-shaped prism moulded onto the end of the waveguide. Back-generated fluorescence is monitored at the light input face of the waveguide. This system is relatively simple to produce by injection moulding techniques.
We constructed a limited reagent assay to measure the binding of fluoresceine (FITC) labelled antibodies specific for human IgG, to the antigen already immobilised to the waveguide surface. The binding curves were completed by 10 minutes incubation without separation of antibody bound from unbound materials. By adding free antigen to the solution phase the binding of FITC antibodies to the surface was competitively inhibited thus producing a dose-response curve.

4 CONCLUSION

We present a novel disposable biosensor for carrying out homogenous, kinetic fluorescence immunoassays. This biosensor will be most useful in the field of medicine.

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