

Immunosensors

Electra Gizeli and Christopher R Lowe

Immunosensors are important analytical tools for monitoring antibody–antigen reactions in real time. Recent developments in immunosensors have produced systems that allow rapid and continuous analysis of the binding event without the requirement for added reagents or separation/washing steps. As a result, great interest has focused on commercializing immunosensors for applications in areas such as clinical, environmental and food analysis.

Address

Institute of Biotechnology, University of Cambridge, Tennis Court Rd, Cambridge CB2 1QT, UK

Current Opinion in Biotechnology 1996, 7:66–71

© Current Biology Ltd ISSN 0958-1669

Abbreviations

BSA	bovine serum albumin
2,4-D	2,4-dichlorophenoxyacetic acid
ELISA	enzyme-linked immunosorbent assay
F1	fraction 1
HSA	human serum albumin
PSA	prostate-specific antigen
QCM	quartz crystal microbalance
SAW	surface acoustic wave
SPR	surface plasmon resonance

Introduction

Immunosensors are defined as analytical devices that detect the binding of an antigen to its specific antibody by coupling the immunochemical reaction to the surface of a device known as a transducer. They have been the subject of increasing interest during the past decade mainly because of their potential application as an alternative immunoassay technique in areas such as clinical diagnostics and environmental control. Regardless of the transducer or application involved, an ideal system would have the following specifications: first, an ability to detect and quantify the antigen, within the required concentration range and a reasonable time (preferably a few seconds); second, a capacity to transduce the binding event without externally added reagents; third, a capacity to repeat the measurement on the same device (i.e. the immunochemical reaction should be reversible); and fourth, the ability to detect the specific binding of the antigen in real samples.

Today, very few systems, if any, possess all of the above properties. Nevertheless, neither the commercial potential nor the importance of immunosensors as an alternative immunoassay technique should be underestimated. The increasing amount of immunosensor-related research published every year, as well as the continuous development of both physical and biochemical aspects of immunosensors, indicate the enormous significance still

attached to this research field. Currently, immunosensors offer scientists a powerful tool for monitoring biospecific interactions in real time and for deriving information about the binding kinetics of the immunoreaction or the structure of molecules and their biological function.

Depending on the transducer technology employed, immunosensors can be divided into three principal classes: optical, piezoelectric and electrochemical. Furthermore, on the basis of the immunoassay format used, they can be either direct (where the immunochemical reaction is directly determined by measuring the physical changes induced by the formation of the complex) or indirect (where a sensitively detectable label is combined with the antibody or antigen of interest). A sufficiently high selectivity may be obtained with non-labelled immunosensors, although non-specific adsorption onto the biorecognition surface remains a major problem for direct systems.

In this review, we discuss the majority of papers relating to immunosensors that have been published over the past 12 months. The detection principle of most systems is mentioned briefly, although more emphasis is given to the immunochemical application in relation to the type of antibody–antigen interaction, surface modification and sensor performance.

Several reviews have appeared in the literature that examine the potential of immunosensors either as systems for clinical analysis [1] and environmental monitoring [2] or as part of the broader area of biosensors and affinity sensors [3–5]. The problems related to the application of immunosensors in clinical diagnostics, with respect to market needs, have also been examined in another general paper [6*].

Optical immunosensors

A variety of optical techniques have been employed to construct immunosensors. The simplest approach involves the detection of immunochemical complexes on the device surface without any labels, whereas more complex approaches rely on the detection of labelled molecules.

Direct monitoring

Optical immunosensors can be used to monitor directly the binding of an antibody to its specific antigen. Recently, interest has been growing in developing optical immunosensors for environmental monitoring. In one case, the physical and chemical aspects of an evanescent wave immunosensor have been described and the potential of the system for pesticide detection examined [7,8]. The sensor utilizes a Mach–Zehnder interferometer, where the change of the intensity of light due to antibody adsorption, in relation to the intensity of the reference light, can

be detected and expressed as an average protein layer thickness. The immunosensing experiment involved the physical adsorption of polyclonal antibodies against human serum albumin (HSA) and the subsequent detection of HSA within the concentration range of 10^{-11} – 10^{-7} M. For bovine serum albumin (BSA), the minimum detectable concentration was estimated to be 3×10^{-11} M. It was concluded that the sensitivity of the sensor should be increased by at least an order of magnitude in order to apply it to the detection of small pesticide molecules.

The direct optical detection of the pesticide atrazine has been realized in another work based on a competitive test format [9**]. The optical system utilized was reflectometric interference spectroscopy, where the interference of two beams reflected at the two interfaces of a thin transparent film is measured using a spectrometer. A thorough investigation, both theoretical and experimental, was undertaken of the instrument and its test performance. The noise, sensitivity, properties of the sample flow and reproducibility of the assay were measured. The competitive assay involved the immobilization of an atrazine derivative on the surface of the device and its subsequent exposure to a monoclonal anti-atrazine antibody pre-incubated with the test sample. A detection limit of 0.25 ppb was reported for an incubation time of 400 s. Unfortunately, this detection limit is still higher than that imposed by European Union regulations (i.e. 0.1 ppb) and, before considering the commercialization of the sensor, both the sensitivity and the laborious chemistry involved for the activation of the device surface should be improved.

One of the most important points in the design of an immunosensor is the choice of an immobilization method that will retain the stability and activity of the bound antibody. Gao *et al.* [10*] have applied a novel immobilization technique to the surface of another direct optical sensor in order to reduce non-specific binding and increase the stability of the sensing surface. A photo-immobilization procedure was applied where BSA derivatized with aryldiazirines was mixed with the immunoreagent, spotted onto the $\text{TiO}_2/\text{SiO}_2$ wave guide layer of an optical grating coupler sensor, dried and then exposed to activating light. The authors chose to photo-immobilize the F(ab')_2 peptidic fragment of a monoclonal antibody raised against the prostate-specific antigen (PSA) in order to detect the binding of PSA. The monoclonal anti-PSA, which competed for different epitopes on PSA than those bound by the F(ab')_2 , was subsequently used to amplify the signal of PSA binding. A linear plot between the signal and the slope of the PSA/anti-PSA binding was obtained for the PSA concentration range of 2 nM to 50 nM. Interestingly, the F(ab')_2 fragments were shown to retain their biological activity after photo-immobilization, whereas non-specific binding was considerably reduced. Immunosensor chips were still active after storage for 1 month at 4°C.

In another study, the optical excitation of surface plasmon resonance (SPR) at a metal dielectric interface has been used to investigate the adsorption of monoclonal mouse and polyclonal sheep IgG to a gold surface, over a concentration range between $0.1 \mu\text{g ml}^{-1}$ and $100 \mu\text{g ml}^{-1}$ [11]. The kinetics of the binding events were studied by monitoring the change in reflectivity at a fixed angle.

The commercial potential of direct optical immunosensors has already been realized, with two evanescent-wave devices (BIAcore™ and IAsys™) now successfully placed on the market. A review of real-time biospecific analysis using the BIAcore™ system, launched by Pharmacia Biosensor (Uppsala, Sweden), has been published [12]. This system is based on a SPR device that measures changes in the refractive index occurring at the carboxy-methylated dextran-activated device/sample interface. Several applications have been reported where not only the concentration of the protein of interest is determined, but also information about its subclass identity, epitope specificity, kinetics, affinity and activity. A similar paper has been published [13] in which biomolecular interactions have been quantified using another commercially available device, IAsys™ (Fisons Applied Sensor Technology, Cambridge, UK). In this paper, carboxy-methylated dextran was attached to the surface of the resonant mirror device onto which proteins were covalently attached. Two immunoassays were carried out. In the first, protein A was immobilized both to detect the binding of IgG within the concentration range of 2–600 nM and to determine the kinetic constants of the reaction. In the second, egg lysozyme was immobilized to study its subsequent interaction with a whole, or fragment of an, anti-lysozyme antibody. A comparative study was presented, where the equilibrium and rate constants of anti-lysozyme binding to lysozyme were determined by IAsys™, BIAcore™ and conventional solution techniques. Results obtained with both optical devices were in good agreement.

Richalet-Sécordelet *et al.* [14**] have recently shown the importance of immunosensors as analytical scientific tools using a standard immunoassay technique and a direct immunosensor to study the cross-reactivity of monoclonal antibodies to a chimeric peptide of HIV-1. In this work, the capacity of monoclonal antibodies to bind to various peptides of different HIV-1 isolates was measured using enzyme-linked immunosorbent assay (ELISA), whereas the dissociation constants and inhibition of binding were measured using the BIAcore™ system. On the basis of the good correlation of the data from the two methods, the authors concluded that the optical immunosensor can be used for selecting peptides of 20–30 residues in diagnostic solid-phase assays.

Indirect monitoring

Many approaches to optical immunosensors are indirect, involving the use of reagents attached to the antibody

or antigen of interest. The requirement for labelled substances makes these sensors less appealing than direct systems in terms of cost and sample preparation; however, the analytical performance of indirect optical immunosensors is, in general, better than that of direct systems both in terms of sensitivity and in terms of reduction of non-specific binding.

Total internal reflection fluorescence has been used to model the equilibrium behaviour of a competitive immunosensor in relation to the physical parameters of a real device [15]. The dissociation constant of the antibody-antigen complex in the immobilized form has been shown to be one of the most significant parameters for the performance of the sensor. In another study, an evanescent wave optical fibre immunosensor was used to determine the kinetic response and absolute sensitivity [16]. Both theoretical and experimental data have been employed to measure the active antibody density on the fibre probe and determine whether the bound antibodies are bivalent or monovalent.

One of the most intensively investigated topics in sensor technology is the regeneration of the biorecognition surface. The feasibility of regenerating the activity of immobilized antibodies on an evanescent wave fibre optic probe has been examined by studying two systems: first, the binding of rhodamine-labelled goat IgG to a polyclonal rabbit anti-goat IgG; and second, the binding of rhodamine-labelled trinitrobenzene to the monoclonal mouse anti-trinitrobenzene IgG [17]. Results show that fibres coated with polyclonal rabbit anti-goat antibody against the large protein retain 70% and 60% of the original signal after five consecutive regenerations with acidic and basic solvent systems, respectively. Fibres coated with monoclonal mouse anti-trinitrobenzene IgG specific for the small organic molecule retained 90% of the original signal when regenerated with basic and ethanolic solutions. The possibility of regenerating the surface would enable the reuse of the same fibre for many tests, thus avoiding the minor differences in response resulting from fibre-to-fibre variation. Obviously, such a device would require recalibration before each sample test.

One of the problems of conventional fluorescence-based immunoassays is the high background signal originating from scattering from the instrument or the sample. This problem has been addressed with the introduction of fluorescence capillary fill device, where three different fluorophores are evaluated as labels for the development of an optical immunosensor against the PSA in serum and whole-blood samples [18]. Compared with the other fluorophores, allophycocyanin gives the best results mainly because its excitation and emission wavelengths are >600 nm, which is the intrinsic fluorescence wavelength of blood. An interesting observation was that the performance of the sensor is considerably improved when whole blood is used instead of serum [18]. This was thought

to result from a decrease in the background noise arising from the fluorescence absorption by cellular components in blood.

A novel application of indirect sensors was reported using a fibre optic immunosensor to detect the fraction 1 (F1) antigen of *Yersinia pestis*, which is the pathogen responsible for plague [19]. Captured antibodies that bind to F1 antigen were immobilized on the core surface of the fibre to form the basis of a sandwich fluoro-immunoassay. The method determined F1 antigen concentrations from 50 ng ml^{-1} to 400 ng ml^{-1} in buffer, serum, plasma and whole blood, with a 5 ng ml^{-1} detection limit.

Piezoelectric immunosensors

Recent advances in transducer technology have stimulated great interest in piezoelectric devices and, today, acoustic wave systems offer the opportunity for direct immunosensing. The sensing technique is based on the measurement of changes of frequency response resulting from adsorption of an antibody or antigen onto a modified piezoelectric crystal surface. There are two main types of piezoelectric device: first, the quartz crystal microbalance (QCM), which operates at frequencies below 15 MHz; and second, the surface acoustic wave (SAW) device, which operates at frequencies normally above 100 MHz. The relationship between frequency shift and surface mass change for the QCM is given by the Sauerbrey equation. More complex equations have been derived for the different types of SAW device. The considerable number of papers that have appeared on piezoelectric immunosensors indicates the potential of these systems as low-cost sensors for one-step detection of immunochemical reactions. Developments in piezoelectric immunosensors and their applications, including the monitoring of antigen binding, cell binding or virus binding to the device surface, have been reviewed recently [20].

The QCM device has been used widely to study the surface modification of the piezoelectric crystal and subsequent detection of the antibody of interest. The simplest application involves measuring the resonant frequency of the device after air-drying the crystal. IgM was detected down to a concentration of 10 ng ml^{-1} with a protamine-activated crystal [21]. Despite the absence of liquid, a discrepancy was found between the calculated and measured frequency change. This was attributed to surface roughness and different viscoelastic and acoustic properties of quartz and deposited layers of mass. In a different study, Dubrovsky *et al.* [22] report the immunological activity of rabbit anti-mouse antibody deposited on differently activated surfaces. The deposition of rabbit anti-mouse antibody using the Langmuir-Schaefer method on a similarly deposited protein A layer gave the best results for the detection of mouse antibody, with a detection limit of 10 pM. In another report, Geddes *et al.* [23] have used QCM to derive information about mass coverage during the self-assembly of a thiol layer on the

gold electrode, subsequent immobilization of IgG and final binding of anti-IgG [23]. These authors observed that the QCM response is not a reliable indicator of surface coverage for small molecules such as thiols; the frequency response associated with IgG immobilization was more consistent with the formation of a packed monolayer of antibody.

The detection of small molecules with the QCM device has been the subject of two papers. In one of these studies, a competitive immunoassay was used to detect atrazine after drying the device [24]. Anti-atrazine was adsorbed on a polystyrene-coated device followed by exposure to a sample solution also containing protein-labelled atrazine. Atrazine concentrations were detectable in the range 0.001 ppb to 1 ppb. This is the lowest detection limit reported using an immunosensor. Apparently, the main reason for this high sensitivity is that measurements were performed after drying the device, a fact that reduces the applicability of this sensor to a portable on-line detector. Unfortunately, the antibody employed was shown not to be specific to atrazine and responded to other triazine derivatives as well.

Liquid-based QCM immunosensors have been used in several applications. A sandwich-type assay for HSA has been reported, where a linear correlation between frequency and HSA was observed within the concentration range of $1 \mu\text{g ml}^{-1}$ to $20 \mu\text{g ml}^{-1}$ [25]. Information about the stoichiometry of conjugates between HSA and anti-HSA was obtained on the basis of frequency measurements during the two-step sandwich assay. Recent studies with a QCM have described the development of an acoustic immunosensor for insulin measurement [26]. The best results were obtained by modifying the surface with protein A and cross-linking it with anti-insulin antibody. Insulin binding was detected within the concentration range of 1 ng ml^{-1} to 0.1 mg ml^{-1} . A mild detergent for removing the bound insulin molecules from the antibody-coated crystal had little effect on the immobilized insulin antibody, demonstrating the capacity of the device for reuse. In another study, monoclonal antibodies against 2,4-dichlorophenoxyacetic acid (2,4-D) have been detected in liquid using a QCM device [27]. The antigen 2,4-D was immobilized on the device surface through an albumin layer. PE measurements were used to investigate the affinity constants of the antibody binding to 2,4-D. A study of particular interest compares the utility of simultaneous operation of a QCM and SPR analysis to assay for HIV antibodies in rabbit sera [28**]. In this paper, the similarities and differences of the physical principles of the two methods are described and experimental data are used to derive the detection limit of each method as a comparable guide parameter. Results show that both systems respond similarly to surface perturbations and assay analyte with a detection limit of the order of 20 nM HIV antibody.

High-frequency SAW-type devices have also been applied to immunosensing. These devices offer higher sensitivity than QCM because mass sensitivity is directly related to the operating frequency. Dahint *et al.* [29] have used an acoustic plate mode device to demonstrate the principle of a high-frequency immunosensor. Recently, we have employed a more complex geometry, the acoustic waveguide device, to detect a peptide sequence (NANP)₆ (one-letter amino acid code) from the protein that coats the malaria parasite (E Gizeli, M Liley, CR Lowe, H Vogel, unpublished data). The (NANP)₆ sequence was self-assembled on the gold-activated device surface through the carboxyl terminus. The binding and displacement of the anti-NANP antibody to and from the activated device surface was monitored and compared with measurement of a peptide with a different sequence. One of the most interesting recent publications has been the detection of atrazine utilizing a surface transverse wave device [30**]. In this work, the high-frequency (250 MHz) operating sensor employed a competitive assay to detect atrazine in the range of 0.06 ppb to 10 ppm. Although the insertion-loss of the device was quite high (~42 dB), a very stable signal was obtained. The surface was successfully regenerated and after 48 cycles over 10 h, only a 30% decrease in the signal response was reported. If the specific binding of atrazine in the presence of other pesticides is realized, this system could offer a good alternative to on-site atrazine detection.

Electrochemical immunosensors

Electrochemical immunosensors have been applied to the investigation of antibody-antigen events through measurements of electrical parameters. The effect of the electrical properties of a buffer on the detection of the immunochemical reaction has been addressed in two papers. In the first, the impedance response of an uncoated sensor in air and buffer solutions of changing ionic strength and pH was measured [31]. It was found that the ionic strength, rather than the pH, has a dominant effect on the sensor impedance. In the second paper, impedance measurements were performed on an amino-silane grafted Si/SiO₂ structure [32]. The hypothesis that each molecular layer is a perfect dielectric has not been verified, indicating the necessity to study not only the capacitive part of the grafted structure, but also the total impedance. It has been suggested that this would become even more obvious in the case of an antibody layer where the size of the molecule enables the formation of a dense well structured layer.

An amperometric immunosensor has also been developed that utilizes an antigen monolayer containing a photo-isomerizable component [33*]. This elegant method makes use of a component that in one of its photo-isomeric states, interacts with the antibody, providing a quantitative measure of antibody concentration. After completion of the measuring cycle, the antigen is photo-isomerized to

state B, leading to release of the antibody. Finally, a screen-printed electrode has been used as an amperometric transducer, together with monoclonal antibodies against 2,4-D (the biospecific element), for detecting 2,4-D using a competitive assay format [34]. A detection limit of 0.1 ppb of free 2,4-D was reported.

Conclusions

Expanding research activity in the area of immunosensors has been amply illustrated in recent literature. The need to develop real working systems, which perform continuously, reversibly and selectively, has now been fully realized, and a considerable amount of work has been devoted to the optimization of sensor performance. A major breakthrough is the establishment of direct optical immunosensors as powerful analytical tools for monitoring immunochemical interactions in real time. Future success in clinical diagnostics will depend on the capacity of immunosensors to both provide the requisite information and meet the demands of a highly competitive market-place.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Aizawa M: **Immunosensors for clinical analysis.** *Adv Clin Chem* 1994, 31:247-275.
2. Marco MP, Gee S, Hammock BD: **Immunochemical techniques for environmental analysis. I. Immunosensors.** *Trends Anal Chem* 1995, 14:341-349.
3. Sharma A, Rogers KR: **Biosensors.** *Meas Sci Technol* 1994, 5:461-472.
4. Leech D: **Affinity biosensors.** *Chem Soc Rev* 1994, 23:205-213.
5. Higson SPJ, Vagdama PM: **Biosensors: a variable monitoring technology?** *Med Biol Eng Comp* 1994, 32:601-609.
6. Connolly P: **Clinical diagnostics opportunities for biosensors and bioelectronics.** *Biosens Bioelectron* 1995, 10:1-6.
An interesting review providing a discussion of the critical issues that need to be taken into account for the successful commercialization of bioimmunosensors. The need for investing in basic research and comparing sensors with routine analytical techniques is emphasized.
7. Schipper EF, Kooyman RPH, Heideman RG, Greve J: **Feasibility of optical waveguide immunosensors for pesticide detection: physical aspects.** *Sensors Actuators B* 1995, 24-25:90-93.
8. Lechuga LM, Lenferink ATM, Kooyman RPH, Greve J: **Feasibility of optical waveguide immunosensors for pesticide detection: chemical aspects.** *Sensors Actuators B* 1995, 24-25:762-765.
9. Brecht A, Piehler J, Lang G, Gauglitz G: **A direct optical immunosensor for atrazine detection.** *Anal Chim Acta* 1995, 311:289-299.
A thorough analysis of an optical atrazine immunosensor is presented. Both the theoretical and the experimental performance of the system are assessed.
10. Gao H, Sanger M, Luginbuhl R, Sigrist H: **Immunosensing with photoimmobilized immunoreagents on planar wave guides.** *Biosens Bioelectron* 1995, 10:317-328.
Reports a novel immobilization technique that is based on the photo-immobilization of the antibody by a photo-linker/polymer-mediated procedure. The regeneration of the immobilized antibody on the surface was successful for 13 cycles, with a coefficient of variation of 6.8%.
11. Geddes NJ, Martin AS, Caruso F, Urquhart RS, Furlong DN, Sambles JR, Than KA, Edgar JA: **Immobilisation of IgG onto gold surfaces and its interaction with anti-IgG studied by surface plasmon resonance.** *J Immunol Methods* 1994, 175:149-160.
12. Lundstrom I: **Real-time biospecific interaction analysis.** *Biosens Bioelectron* 1994, 9:725-736.
13. Yeung D, Gill A, Maule Ch, Davies RJ: **Detection and quantification of biomolecular interactions with optical biosensors.** *Trends Anal Chem* 1995, 14:49-56.
14. Richalet-Secordel P, Zeder-Lutz G, Plaue S, Sommermeyer-Leroux D, Van Regenmortel MHV: **Cross-reactivity of monoclonal antibodies to a chimeric V3 peptide of HIV-1 with peptide analogues studied by biosensor technology and ELISA.** *J Immunol Methods* 1994, 176:221-234.
This paper gives an excellent example of the significance of optical immunosensors as analytical tools. Binding information and kinetic information concerning the cross-reactivity of monoclonal antibodies to a synthetic peptide are derived from ELISA data and BIAcore™ measurements, respectively.
15. Domenici C, Schirone A, Celebre M, Ahluwalia A, De Rossi D: **Development of a TIRF immunosensor: modelling the equilibrium behaviour of a competitive system.** *Biosens Bioelectron* 1995, 10:371-378.
16. Feldman SF, Uzgiris EE, Murray-Penney C, Gui JY, Shu EY, Stokes EB: **Evanescence wave immunoprobe with high bivalent antibody activity.** *Biosens Bioelectron* 1995, 10:423-434.
17. Breslin K, Anderson G, Shriver-Lake I, Ligler FS: **Regeneration of immobilized antibodies on fiber optic probes.** *Biosens Bioelectron* 1994, 9:585-592.
18. Daniels PB, Fletcher JE, O'Neill PM, Stafford CG, Bacarese-Hamilton T, Robinson GA: **A comparison of three fluorophores for use in an optical biosensor for the measurement of prostate-specific antigen in whole blood.** *Sensors Actuators B* 1995, 26-27:447-451.
19. Cao LK, Anderson GP, Ligler FS, Ezzell J: **Detection of *Yersinia pestis* fraction 1 antigen with a fiber optic biosensor.** *J Clin Microbiol* 1995, 33:336-341.
20. Suleiman AA, Guilbault GG: **Recent developments in piezoelectric immunosensors.** *Analyst* 1994, 119:2279-2282.
21. Raman-Suri C, Raje M, Mishra GC: **Determination of immunoglobulin M concentration by piezoelectric crystal immunobiosensor coated with protamine.** *Biosens Bioelectron* 1994, 9:535-542.
22. Dubrovsky T, Tronin A, Dubrovskaya S, Vakula S, Nicolini C: **Immunological activity of IgG Langmuir films oriented by protein A sublayer.** *Sensors Actuators B* 1995, 23:1-7.
23. Geddes NJ, Paschinger EM, Furlong DN, Caruso F, Hoffmann CL, Rabolt JF: **Surface chemical activation of quartz crystal microbalance gold electrodes-analysis by frequency changes, contact angle measurements and grazing angle FTIR.** *Thin Solid Film* 1995, 260:192-199.
24. Yokoyama K, Ikebukuro K, Tamiya E, Karube I, Ichiki N, Arikawa Y: **Highly sensitive quartz crystal immunosensors for multisample detection of herbicides.** *Anal Chim Acta* 1995, 304:139-145.
25. Sakai G, Saiki T, Uda T, Miura N, Yamazoe N: **Selective and repeatable detection of human serum albumin by using piezoelectric immunosensor.** *Sensors Actuator B* 1995, 24-25:134-137.
26. Raman-Suri C, Jain PK, Mishra GC: **Development of piezoelectric crystal based microgravimetric immunoassay for determination of insulin concentration.** *J Biotechnol* 1995, 39:27-34.
27. Skladal P, Minunni M, Mascini M, Kolar V, Frank M: **Characterization of monoclonal antibodies to 2,4-dichlorophenoxyacetic acid using a piezoelectric quartz crystal microbalance in solution.** *J Immunol Methods* 1994, 176:117-125.
28. Kosslinger C, Uttenhaler E, Drost S, Aberl F, Wolf H, Brink G, Stanglmaier A, Sackmann E: **Comparison of the QCM and the SPR method for surface studies and immunological applications.** *Sensors Actuators B* 1995, 24-25:107-112.
Reports a comparative study of the two most widely used direct immunosensors. The theoretical differences and similarities of SPR and QCM sensors are presented. Experimental results show that neither system is superior.
29. Dahint R, Grunze M, Josse F, Renken J: **Acoustic plate mode sensor for immunochemical reactions.** *Sensors Actuators B* 1994, 66:2888-2892.
30. Tom-Moy M, Baer RL, Spira-Solomon D, Doherty TP: **Atrazine measurements using surface transverse wave devices.** *Anal Chem* 1995, 67:1510-1516.

This paper is the first successful attempt to use a high frequency piezoelectric device for detecting low molecular weight analytes. Atrazine, within the concentration range 0.06 ppb to 10 ppm, is detected using a competitive assay; regeneration of the surface after 48 cycles is demonstrated.

31. Hardeman S, Nelson T, Beirne D, Desilva M, Hesketh PJ, Jordan-Maclay G, Gendel SM: **Sensitivity of novel ultrathin platinum film immunosensors to buffer ionic strength.** *Sensors Actuators B* 1995, 24-25:98-102.
32. Schyberg C, Plossu C, Barbier D, Jaffrezic-Renault N, Martelet C, Maupas H, Souteyrand E, Charles MH, Delair T, Mandrand B: **Impedance analysis of Si/SiO₂ structures grafted with biomolecules for the elaboration of an immunosensor.** *Sensors Actuators B* 1995, 26-27:457-460.
33. Willner I, Blonder R, Dagan A: **Application of photoisomerizable antigenic monolayer electrodes as reversible amperometric immunosensors.** *J Am Chem Soc* 1994, 116:9365-9366.
 • This short paper presents an elegant method for regenerating the sensors surface. A photoisomerizable monolayer deposited on the electrode is switched from an immunoactive form A to the inactive form B, after illumination of the surface. Further irradiation of form B restores the activity of the substrates.
34. Kal BT, Sklad LP: **A disposable amperometric immunosensor for 2,4-dichlorophenoxyacetic acid.** *Anal Chim Acta* 1995, 304:361-368.